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THE ORIGIN OF VARIATIONS

SYMPOSIUM AT THE THIRTY-NINTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF NATURALISTS, TORONTO, DECEMBER 29, 1921

VARIATION IN UNIPARENTAL REPRODUCTION

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DARWINISM left the origin of variations the unsolved problem. Give us inherited variations, it said, and we can explain adaptation, by natural selection. But this was the omission of 99 per cent., if not 100 per cent., of the problem of evolution. Are we in better case to-day? Has the experimental study of genetics given us some solid knowledge of the origin, the causes, of variation? Have we learned that the obvious differences observable everywhere among individuals are the foundations of evolution? Or that they are not? Are slight quantitative fluctuations the material out of which evolution is made? Have we discovered that extensive saltations are the steps in evolution? Or that less extensive mutations, qualitative or chemical changes, that may be minute or large, are that by which evolution is constituted? Do we know the origin of such saltations, mutations? Have we found that the present constitution of the organism predetermines in some way the course of further change; or that an *élan vital* is driving the organism to unfold in a definite way, like a flower; that evolution is orthogenesis? Do we comprehend the nature and causes of such a push to unfold, and of the direction in which it tends? Have we found perhaps, as at least one investigator maintains,

that it is mixing of stocks, hybridization, that is the origin of organic diversity? Or do we know that the physical and chemical conditions of the environment produce changes that are inherited and give us evolution? Or finally, do several or all of these methods of action concur?

Such are the questions, I take it, on which we hope for light in the discussion this afternoon, and in the discussion of orthogenesis before the American Society of Zoologists, and of the species concept before the Botanical Section of the American Association.

The lines of attack on the problem of variation are as manifold as are the questions to be answered. The basic idea in that attack whose results I shall try to summarize is this: In the reproduction from two parents familiar to us in higher animals and plants, there is a mixing of different stocks, a formation of great numbers of diverse groups of the hereditary materials, with consequent production of a great variety of diverse offspring from a given pair of parents. This is the chief cause of the differences everywhere observable among individuals: differences formerly classed as variations and considered the material of evolutionary change. But such kaleidoscopic regrouping of materials, the units of which are not changing, has no obvious relation with evolutionary variation; in the next generation a new grouping of the same material occurs, and so on indefinitely. If there likewise occur progressive evolutionary changes, these are so lost, so hidden, in the multitude of kaleidoscopic recombinations that they can not be distinguished; the literature of evolution is filled with confusion due to this difficulty.

Therefore the idea suggests itself: Why not avoid at once all this, by studying evolutionary changes in those organisms where no mixing of stocks is occurring; where there is no kaleidoscopic regrouping of the hereditary materials? There are organisms that reproduce from a single parent, with no shifting or recombination of the germ plasm; in these, actual changes that persist from

generation to generation, such as evolution requires, should lie open before us, unconfused. We should see evolution occurring, as we see water flowing.

This plain, simple and optimistic maxim has, I fear, like many another such, not proved so illuminating as its promise. But led by it, investigators set themselves at the study of the passage of generations, with selection and propagation of individuals showing diversities, in these creatures where seemingly all lasting change from parent to offspring must be evolutionary. Their hope was to see evolution occurring. And what did they see? I need not review the details; Johannsen, Barber, Hanel, the present writer, Lashley, Agar, and others, followed for long periods the passage of generations in many different organisms during uniparental reproduction.

Their report, after years of work, was astonishingly simple and clear. As to the origin of hereditary variations, it resembled the famous chapter on the Snakes of Ireland. It summed itself, in effect, in the succinct, sufficient, exhaustive proposition that there is no inherited variation; hence no origin of such variation. There is nothing to find out about it, for it doesn't occur. The individuals produced in uniparental reproduction may indeed differ, but these diversities are transitory effects of environmental differences; they are not inherited. All the descendants of a single individual are genetically and hereditarily alike; they form in effect a set of identical twins. And from this it could be concluded that in biparental reproduction *all* the observed diversities are due to the kaleidoscopic regrouping of hereditary materials; nothing to evolutionary change.

Outcries—objurgations and acclamations—greeted these propositions. Some reviled them for their manifest absurdity, others acclaimed them for their obvious truth and the clarification they wrought. Opponents tried to disprove them by investigating the matter themselves; their evidence strengthened the propositions they

had thought to overthrow; who came to scoff remained to mourn.

Such was, in the gross, the upshot of the first phase of the study of uniparental inheritance; of perhaps the first ten years. But the matter could not rest here. This work cleared the ground. It showed that 99 per cent. or more of what had been called variation had nothing to do with evolutionary change—a conclusion which Mendelian study was reaching independently. Now it remained to accept that fact, to take a new hold, to grapple with the more difficult question: Is there yet an infinitesimal residuum of evolutionary change? If we select the most favorable organisms, and study them in most minute detail for sufficiently long series of generations, shall we indeed find that there are no persistent variations whatever? Such is the work that has in this field occupied, with redoubled intensity, the last ten years. What are the results of this second phase of the work?

Some of the workers devoted themselves to observational breeding work on the passage of many generations, accompanied by selection; others attempted to modify the inherited characters by physical and chemical agents. In the observational search for persisting alterations, with the attempt to accumulate their results by selection, we find, first, that many of the organisms studied have as yet defied all attempts to find any inherited variations. Such is the report of Ewing on his extended work with aphids; such is the case with the fungi studied by Brierly (1920). Such is the case with most of the strains of the infusorian *Paramecium*, studied in detail for long periods by many different observers. Only in certain deformed strains, and possibly in one or two other instances, has the occurrence of persisting variation been observed in animals living under the usual conditions. Such is the case with the great majority of the strains of the Cladocera studied with such extraordinary thoroughness for long periods by Banta (1921); out of 16 strains to which selection was applied for many generations, all but one

gave on the whole negative results; they did not change. Some of the investigators still insist that this is indeed the outcome of all this work; that all cases seeming to give other results are for one reason or another deceptive; that no hereditary variations occur; that evolutionary change has not been observed in this sort of reproduction—and presumably therefore in no other sort. Thus, for example, argue Brierly (1919), and, in effect, Victor Jollos (1921).

On the other hand, in some of the organisms studied, visible changes persisting from generation to generation of uniparental reproduction have been observed. Even in the first period of this sort of work, extremely rare "mutations" were reported by Barber in his work on bacteria, an apparent single one by Lashley in *Hydra;* a "bud variation" or two by Johannsen; and other isolated cases occurred. In the second period of the work, as a matter of observational fact, whatever the interpretation, it is certain that in the lowest Rhizopoda: in *Difflugia,* in *Centropyxis,* in *Arcella;* in the infusorian *Stylonychia,* and in certain abnormal strains of *Paramecium,* as studied in our laboratory at the Johns Hopkins University, there arise in uniparental reproduction, changes affecting both physiological and structural characters; changes that may be very slight, or of great extent; that are passed on to later generations in uniparental reproduction. By selection and breeding of the changed individuals, stocks are isolated which differ persistently from the stock with which the work of breeding began. In this way might well arise the diverse biotypes found in nature to occur within a species, in these organisms. Something similar was found by Stout in the propagation of certain plants by cuttings.

Again, among the 16 strains of Cladocera, subjected by Banta to selection for a physiological characteristic, one, and only one, showed persisting alterations, accumulated by the selective process, so that from the single strain, two continuously diverse strains were produced. Jollos

too has observed a few cases in which strains of *Paramecium* became differentiated in ways that could hardly be considered the result of environmental action. Doubtless some other cases might be collected. Here then we seem to have what we were searching for; here at last is something solid; here by our presuppositions we have evolution evolving; we have *seen* it! But as with so many of the seeming solid things of science—so these became sicklied o'er with a pale cast of thought, of doubt, of speculation. What, it is asked, is the cause, the fundamental nature, of these persistent changes? And are they indeed of a sort to be considered steps in evolution?

And when we look closely, the observational and selectional work has given us little information on these points. In *Arcella* Hegner found that certain of the inherited structural changes are mere results of increase or decrease in number of nuclei, brought about in a simple manner. But most of the changes in the lower organisms studied can not be accounted for in this way. The work of Erdmann (1920) indicates that certain persistent changes occur in *Paramecium* as a result of the periodic nuclear reorganization called endomixis. These would perhaps have only a significance similar to that of the recombinations occurring in biparental inheritance. It is a favorite speculative idea with opposing speculators that most or all of the persisting changes we have mentioned arise through irregularities in nuclear division, and hence are of little evolutionary significance, but this is thus far a mere possibility, without solid base; as the Germans say, it floats in the air. Another speculative notion is that the changes lack permanence; that if followed for a sufficiently great number of generations there would be reversion to the original condition. Whether this doubt can ever be resolved by observation can not be predicted; it depends perhaps on the number of generations demanded by the doubters.

In the attempts to modify inherited characters by physical and chemical agents, more positive evidence as

to the cause of variation has perhaps resulted. Can we not, it is asked, by subjecting the hereditary material to chemicals, to physical agents, alter it, as we can alter practically everything else in nature? Of course we can; it is easy. But when we alter it we usually kill it, or prevent it from developing; our task is like that consummation devoutly to be wished, of killing the pathogenic bacteria in a man—which is easy—but it also kills the man! Have we succeeded in so altering the germ plasm, without killing it, that it now develops differently, and transmits the diversity to its progeny?

It is easy by altering the chemical and physical conditions to change tremendously the development and characteristics of these creatures, and that without stopping life and reproduction. But in the infinitely greater proportion of cases such changes have no inherited effect; so soon as these particular conditions are removed, the progeny go back at once to the usual constitution. Such has been the result of extensive experiments of my own in modifying *Paramecium* with chemicals; and of Noyes in modifying Rotifera. Startling transformations of form, structure and function are readily produced and kept up for generations, but disappear when the offspring are reared under normal conditions. Once in our work the task seemed accomplished. After many generations of treatment with alcohol, *Paramecium* yielded monstrosities and deformities, analogous to those Stockard obtained by the same method in guinea pigs, and these deformities were transmitted after removal from alcohol, for generation after generation. This was stirring; all the energy of the laboratory was devoted to following the monstrous stock through long periods, leaving the formulation of pedigrees till time permitted. But when this could be done it appeared that all these abnormal individuals came from one single ancestor, out of the hundreds with which the experiment began; the rest had all returned at once to normal. We know that such hereditarily abnormal stocks occur at times in *Paramecium,*

produced in some frequency by an agency which takes the matter at once out of the field with which I am dealing —by the recombinations occurring at conjugation, at biparental reproduction. Our monstrous stock may have come from such an individual, included by accident in the experiment. Our spirit-stirring results faded into nothingness—a type of what has so often happened in promising work in the inheritance of environmental effects, of what will probably often happen again.

Other workers have been more successful. In the bacteria, if we can accept the accounts given by many investigators, and well summarized, for example, in Adami's "Medical Contributions to the Theory of Evolution," environmental conditions frequently alter, in an adaptive way, the persisting characteristics of the stocks, differentiating a single race into several. The difficulties of certainly working with unmixed strains is very great in these minute creatures, a fact which leads many students of experimental evolution to reject generalizations based on these organisms. Further, the extraordinary work of Löhnis (1921), recently published by the National Academy, tends, if substantiated, to so completely upset all supposed knowledge of life history in the bacteria that it will be best to omit these from consideration until the air is cleared. For similar reasons, and from considerations of space, I will not speak of the work on pathogenic Protozoa.

Turning then to those larger organisms that are isolated with as much ease as are guinea pigs, Middleton has found that differences of vigor and of rate of reproduction are produced by subjection of infusoria for long periods to diverse temperatures, and are perpetuated, after equalizing the temperatures, from generation to generation for long periods, and through the process of conjugation. At this meeting he has reported similar results produced by subjection to diverse chemicals. How far this is comparable to change of other characteristics than reproductive vigor we do not know.

Victor Jollos (1921) has just published in this field work which must make a deep impression on the study of experimental evolution, work which gives us more positive results than have before been achieved. By experimentation extending over years he has, by subjection for long periods of time, altered the resistance of the infusorian *Paramecium* to certain chemicals, and to heat. After removal of the causative agent these physiological changes are passed on from generation to generation of uniparental reproduction, for longer or shorter periods. Of extreme interest is the fact that longer subjection to the altering agent causes longer persistence after the agent is removed. The induced changes lasted in some cases for hundreds of generations, not yielding at the periodic nuclear reorganizations known as endomixis. But the acquired resistance in practically all cases finally disappeared if the organisms were continued sufficiently long in the normal conditions. Subjection to frequently varied environment hastened the disappearance of the persisting effect; and it usually disappeared at once when there occurred the profound reorganization accompanying conjugation and biparental reproduction. But in some cases, as in Middleton's results, the acquired resistance lasted through conjugation; even through several cycles of conjugation. But in all cases in which it was clear that he was dealing with resistance acquired through subjection to chemical or physical agents, it finally disappeared, after hundreds of generations, if the organisms were kept sufficiently long in an environment lacking the causative agent. Jollos is from this inclined to draw the conclusion that the changes are not comparable to the (assumedly) permanent differences that separate genotypes or species, and hence that they do not indicate a method by which such permanent differences may arise.

Here emerges an obvious logical difficulty involved in all work on the production of inherited change through environmental action. If we succeed in producing such

change, it is clear that the character altered was not a permanent one. And if after long re-subjection to the original environment the induced change disappears, it is equally clear that the new character was no more permanent than the original one. If we now assume that there are other characters that *are* permanent, not alterable by environmental action, of course we can obtain no light on these by changing those characters that can be changed. To me it appears that we have no right to assume, at the present stage in the game, that any such absolutely permanent characters exist. If this be true, then the production of changes persisting through many generations of uniparental, and even of biparental, reproduction, with the further fact that the greater the number of generations the altering agent has acted, the greater the number of generations the change persists, seems of the greatest interest. It perhaps would, if action of the environmental agent continued sufficiently long, lead to production of inherited characteristics that are as permanent as any such characters are. It is certainly, as Jollos agrees, capable of producing such diversity of biotypes as we find within a species; and it might perhaps, if the results of diverse agents are cumulative, produce any of the inherited diversities found in organisms. This is the most promising lead that we have found in the study of uniparental production.[1]

In sum, the study of variation in uniparental reproduction yields the following: The germinal or genotypic constitution in most organisms is extremely stable; in many stocks it changes not at all, so far as observation goes. To alter it by physical or chemical agents is usually to kill it. In some of the lowest organisms—rhizopods, bacteria, some infusoria—it changes with somewhat greater frequency, though still rarely. The nature of the changes, and whether they may be permanent, or must after many generations revert to the original condi-

[1] The important observations and discussions of Jollos relating to changes producible by environmental action at the time of conjugation do not fall within the compass of a discussion of uniparental reproduction.

tion, is in some dispute. In these same organisms, environmental agents may produce changes persisting through many generations of uniparental reproduction and even through biparental reproduction, the period of persistence depending partly on the number of generations through which the producing agent acted. This suggests that inherited characters as permanent as any that exist might in time be so produced. In spite of important differences of opinion among investigators, to the reviewer the facts in uniparental reproduction seem to point more toward the production of evolutionary change by the action of the environment on the germ plasm than by any of the other methods. In this respect it takes its place in that modern revival of work on the inheritance of acquired characters, of which we had so striking an example this morning, in the account of the dizzy rats and of the inheritance of their dizziness; though in the study of uniparental reproduction nothing has appeared that indicates a transfer of somatic characters to the germ.

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VARIATIONS IN DATURA DUE TO CHANGES IN CHROMOSOME NUMBER

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Two forms with which we have recently carried on breeding experiments, the garden flower *Portulaca* and the jimson weed (*Datura Stramonium*), are strikingly different in the types of variations which they show. The *Portulaca* is procurable in a wide range of color varieties, and is apparently subject to relatively frequent mutations, both seminal and somatic, with sectorial and periclinal chimeras a common phenomenon. Sufficient breeding tests have been made to indicate that the varieties of *Portulaca* are due in large measure at least to gene mutations. In comparison with *Portulaca,* the jimson weed is relatively stable so far as gene mutations are concerned. Despite the large amount of breeding work with this species, both before and since the rediscovery of Mendel's law, only the two allelomorphic pairs of characters, purple *vs.* white flowers, and spiny *vs.* smooth capsules, have been identified aside from the pair, tall *vs.* short stature recently determined by the writer and Avery (3).

It is true that certain of our pure lines of *Datura* differ slightly from others when grown in comparable pedigrees, but the fact remains that so far as sharply contrasting Mendelian characters are concerned, the jimson weed is highly stable, while the *Portulaca* is highly mutable. Our knowledge of changes in chromosome number in other forms is not sufficient to indicate if there is any significance for the present discussion in the difference just mentioned between *Portulaca* and *Datura.*

Our interest in *Datura* began about 1910 or 1911, when the jimsons were used as demonstration material for students in genetics. In 1915 we found our first mutant which we called the Globe from the shape of its capsules.

The capsules of normal plants are ovate and the edges of the leaves somewhat toothed. Globe plants, on the contrary, have depressed capsules and broader leaves with a more entire margin (cf. 3, figs. 7 and 9). Figure 1 shows

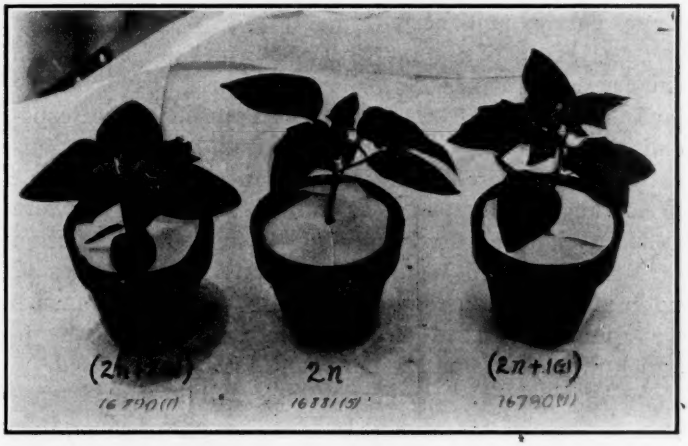

FIG. 1. Young plants in 3-inch pots. The normal $2n$ plant is in the middle, the $(2n+1)$ Globe on the right, and the $(2n+2)$ Globe on the left.

young plants beginning to flower. In the center is a normal and on the right a Globe. The leaves of the latter are broader and more closely massed together. In the plant on the left, the Globe characters are more strongly developed. This plant represents an extreme type of the Globe mutant, and has been called the Round-leaf Globe. It is of considerable genetic interest and will be discussed later. It was at first thought that the Globe might be a tetraploid type like the Gigas *Œnothera* but a preliminary cytological investigation showed that such was not the case.

A peculiarity in the inheritance of the Globe (1, table 3) was found to be that the Globe complex is transmitted to only about one fourth of its offspring when a Globe parent is selfed; that about the same proportion of one fourth Globes only appears in the offspring when the Globe parent is crossed with pollen from a normal plant; and that the mutant character is transmitted to only a slight

extent or not at all through the pollen—to less than 2 per cent. in a large series of crosses.

The next mutant found was Cocklebur (3, fig. 11) named from the resemblance of its fruits to those of the cocklebur weed. The plant is weak and lopping and the leaves narrow and twisted.

The Poinsettia mutant (3, fig. 14) was named from a fancied resemblance of its long clustered leaves to the hothouse plant of that name. The Poinsettia is of especial interest, since this mutant was found to give curious ratios when heterozygous for color factors.

As our eyes became better trained, other mutants were added to the list, largely through the keen discrimination of Mr. Avery and Mr. Farnham, until we now have 12 main mutants with some varieties, all of which transmit their mutant characters essentially in the same way in which the Globe complex was found to be transmitted.

In addition we had a mutant which, unlike the 12 types just mentioned, was found to breed true, and since it is practically impossible to obtain crosses between it and the normal form from which it arose, it was called "New Species" (3, fig. 15). The capsules are somewhat spherical and the leaves broad, although in a race of the same type later discovered the leaves are not greatly different from the normals. Heterozygous plants of the "N. S." sometimes gave curious ratios in their offspring.

Such was the situation up to the spring of 1920, when we were fortunate in securing the cooperation of Mr. Belling in a study of the nuclear condition of our mutants. On the basis of his work we are able to make the classification of types shown in Fig. 2. In the individual figures—which of course are highly diagrammatic—the chromosomal constitution of somatic cells is represented. We have not attempted to represent the size differences determined by Mr. Belling and pictured in our paper in the morning session.[1] A word of explanation of terms is desirable. The terms diploid, triploid and tetraploid are already current to indicate a balanced condition in which each chromosomal set (we can not say chromosomal

[1] To be published shortly in the AMERICAN NATURALIST.

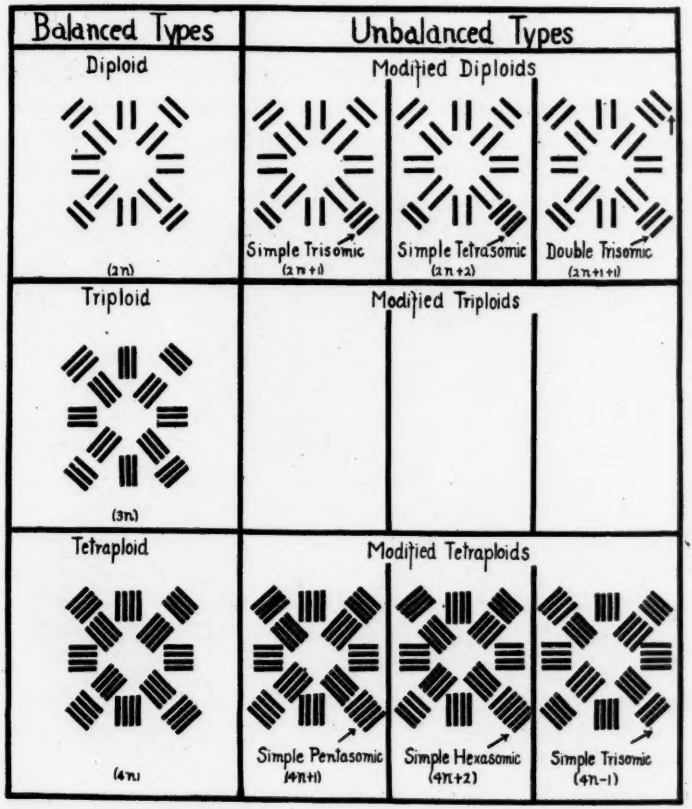

FIG. 2. Diagrams illustrating the chromosomal types already found in *Datura*.

pairs when there are more than 2 in a set) has respectively 2, 3, or 4 chromosomes. I have suggested (2) the terms disome, to indicate a set of 2 chromosomes, trisome a set of 3, and tetrasome a set of 4, etc., with the adjectives disomic, trisomic, tetrasomic, etc. Such terms may be found useful, but it seems impossible to devise a simple terminology that will adequately describe even the chromosomal irregularities at present known in *Drosophila* and *Datura*. Accordingly, after considerable discussion with Dr. Bridges, we have agreed upon a set of formulæ which is illustrated in the diagram and which we shall use in our present papers.

Of the balanced forms there are even-balanced or stable, and odd-balanced or unstable types. In the even-balanced diploid, which is the normal jimson weed, the two chromosomes in each set go to opposite poles by the ordinary process of disomic reduction, and the plants breed true for chromosome number. Partly for the same reason, the even-balanced tetraploid, which is our "New Species," breeds essentially true. The triploid, on the other hand, is odd-balanced and therefore unstable, since in the trisomic disjunction in each set two of the three chromosomes go to the one pole and one to the other, the process taking place at random. Through the operation of chance, therefore, gametes of different chromosomal number will be formed, and simple and double mutants as well as diploids will occur in the offspring. The relation may be seen from the pollen of the three balanced types under the same magnification (Fig. 3), where the photograph at the left (*a*) shows a field of pollen from a diploid; that at the right, (*c*) with larger grains, pollen from a tetraploid; while that above (*b*) shows pollen from a triploid. Pollen from a triploid is not only characterized by a large proportion of empty grains, but also by a great diversity in the size of the grains brought about by the differences in the number of chromosomes which they contain.

The upper left-hand figure of the unbalanced types (Fig. 2) has one extra chromosome in the lower right-hand set, indicated by the arrow, giving 1 trisome, and 11 disomes in this nucleus, and its formula may be written $(2n+1)$. Such a simple mutant is the Globe—simple because only one set is affected. If another set has the extra chromosome—say the set on the right—instead of the one with the arrow, this extra chromosome would cause the plant to assume the characters of, say, the Cocklebur mutant. It is obvious that since there are 12 sets in *Datura* and each set may have an extra chromosome, there are 12 mutants with the formula $(2n+1)$ theoretically possible. Through the process of disjunction in these 12 mutants, half of the gametes should contain the extra chromosome,

and half should not. Differential mortality, affecting adversely zygotes with the extra chromosome, prevents the expected equality of $(2n)$ and $(2n+1)$ individuals in the offspring from test crosses with diploids.

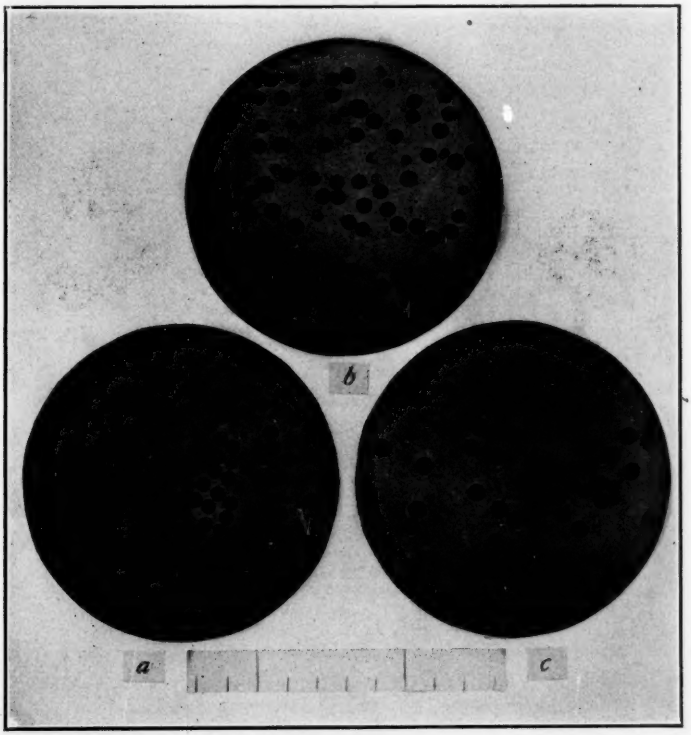

FIG. 3. Photomicrographs of pollen grains: (*a*) from a diploid *Datura*; (*b*) from a triploid; (*c*) from a tetraploid. The magnification is indicated by the scale, each division of which equals 0.10 mm.

The 12 mutants under discussion may best be represented in a single figure by their capsules. In Figure 4 we have capsules of the 12 simple trisomic mutants viewed from the ovate side, each one of which represents the addition of a single extra chromosome presumably in a different set. There is the Globe with depressed capsules and stocky spines; the large long-spined Poinsettia; the narrow short-spined Cocklebur; the slender-spined Ilex;

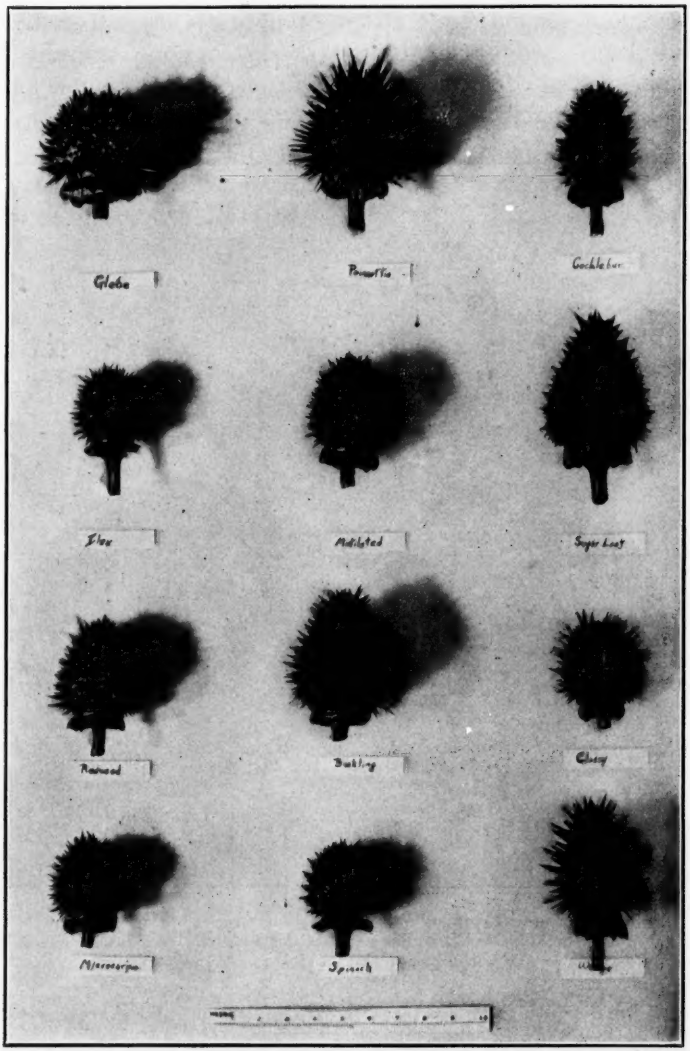

FIG. 4. Photographs of capsules of 12 mutants of *Datura* viewed from the ovate side.

the Mutilated, usually mutilated with a diseased blotch; the short-spined Sugarloaf; the shiny capsule of Glossy, etc., with lastly the narrow, long-spined Wedge. I have

provisionally called these mutants the 12 apostles. Certain of the 12 have varieties which may be called acolytes, and perhaps some of these in the figure may be reduced from the rank of apostles to that of acolytes when other forms are discovered.

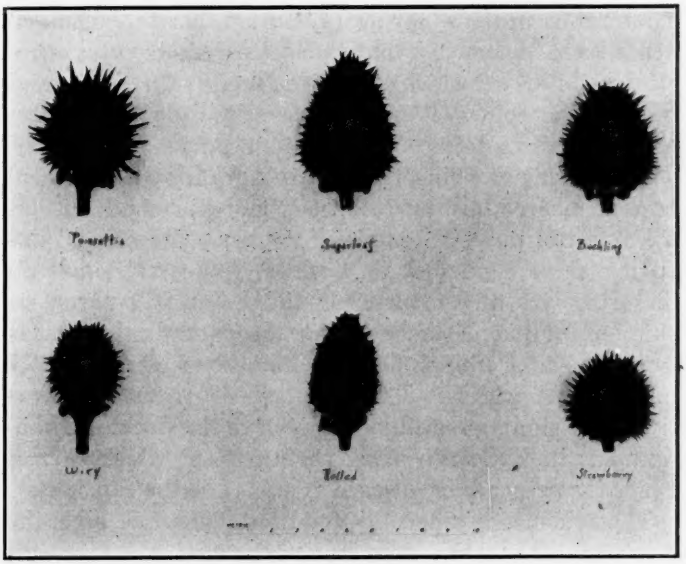

FIG. 5. Capsules representing 3 pairs of mutants. Those in the lower row are believed to represent varieties or "acolytes" of the respective types represented above.

In Figure 5, the mutants from which the capsules in the lower row were taken have been provisionally classed as acolytes of their respective apostles represented above. The evidence is best in regard to the mutants Wiry and Poinsettia which form the pair at the left in the figure. They both contain a single extra chromosome of approximately the same size, and in both cases this extra chromosome is shown, by peculiar color ratios in their offspring, to be in the set which carries the factors for purple and white flower color. The fact that though perfectly distinct they are yet similar in appearance, and the fact that one has not infrequently given rise to the other in our

cultures, is a line of argument applicable not alone to the pair Wiry and Poinsettia. It also leads us to consider Rolled an acolyte of Sugarloaf, and Strawberry an acolyte of Buckling. The possibility of acolytes being caused by modifying Mendelian factors is being investigated.

I have said that the Poinsettia mutant gave curious color ratios in its offspring (4 and 2, table 2). The evidence seems conclusive that Poinsettia has its extra chromosome in the set which carries the factors for purple and white flower color. A heterozygous Poinsettia may have one dose or two doses of the dominant purple flower color. The offspring of Poinsettia (like those of the Globe), it will be remembered, are part normals and part mutants. If a Poinsettia parent is duplex for purple, its normal offspring show 8 purples to 1 white, while its Poinsettia offspring are all purples. If the Poinsettia parent is simplex for purple the ratio for the normal offspring is 5 purples to 4 whites, and for Poinsettia offspring is 7 purples to 2 whites. The back crosses are also distinctive. By similar reasoning we believe the Cocklebur mutant has its extra chromosome in the set which carries genes for presence or absence of spines on the capsules.

The evidence is especially good for Poinsettia, since the color classes can be recognized in the seedpan. Using a Poinsettia which arose in a purple line from Washington, D. C., we crossed it with a white line of similar appearance also from Washington, and, without going outside of these two lines, have synthesized Poinsettias of all the possible combinations of color factors and have made nearly all the possible combinations of crosses between them. The results with the Washington lines are in accord with what would be expected from a random assortment of 3 chromosomes in the set containing the purple-white color factors. In a certain group of Poinsettias simplex for purple in which the 2 chromosomes bearing the white factor might have been brought in, so far as we knew, either from the white Washington stock, or from a distinct white line from Erfurt, Germany, the color ratios in the offspring of some parents were according to calcu-

lation, but from other parents the whites were approximately 6 times as frequent as would be expected. Later experiments seem to indicate: that we get the definite excess in white offspring from simplex parents when both the "white" chromosomes come from the German line; that we get the Poinsettia ratios typical of random assortment when the two white chromosomes come from the Washington whites; and that we get both of the two types of ratios from different individual F_2 parents when we make up an F_1 Poinsettia containing both a Washington white, and a German white chromosome. It is apparent that the peculiarity must be attributed to the German chromosomes. The question is receiving further experimental investigation but our provisional hypothesis to account for the difference in the ratios is that for some reason in trisomic disjunction the German white chromosomes go to opposite poles rather than to the same pole 6 times as frequently as the laws of random assortment would dictate.

Let us return to our diagrams in Fig. 2. Of the modified diploids we may have 2 extra chromosomes in a single set forming a simple mutant of the formula $(2n+2)$. An example is the round-leaf Globe (fig. 1) already mentioned.

If two different sets are affected each with a single extra chromosome we have a double mutant with the formula $(2n+1+1)$. Of the 66 different double trisomic mutants theoretically possible, we have a considerable number now under cultivation. As an example, the double mutant Globe-Reduced is shown in Fig. 6. At the top is a capsule of a normal diploid with its chromosomal diagram. At the left is a capsule of the Globe, and at the right a capsule of Reduced. Their diagrams indicate that the two mutants have different sets affected. The plant represented by the capsules below, from the appearance of its leaves as well as from that of its fruit, is undoubtedly a double mutant with the two sets affected as indicated in the diagram below. If the Globe-Reduced behaves like other double mutants we have bred, its off-

spring should contain normal diploids, both the Globe and the Reduced mutants, as well as the double mutant, Globe-Reduced, roughly in the proportion of 6: 2: 2: 1.

Triploids (fig. 2) have been discussed in this morning's session. Our prediction at last year's meeting has been

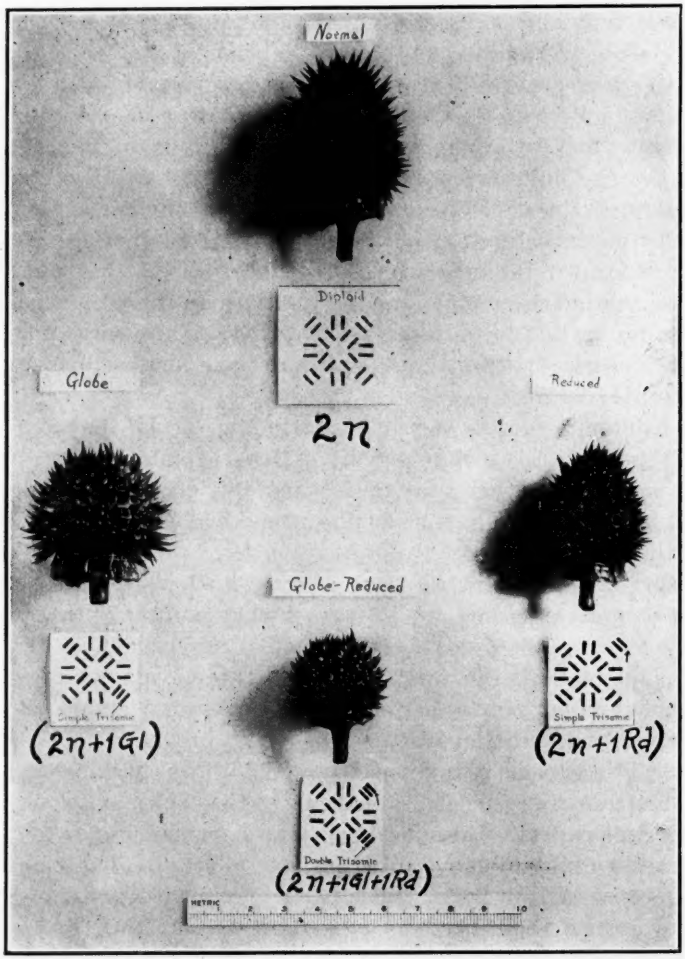

FIG. 6. Capsule of normal diploid ($2n$) above; capsule of Reduced ($2n+1$ *Rd*) at right; capsule of Globe ($2n+1$ *Gl*) at left; and capsule of double mutant Globe-Reduced ($2n+1$ *Gl*$+1$ *Rd*) below. Below each capsule is given its chromosomal diagram.

fulfilled and we have obtained, in the offspring of a triploid, practically the full range of $(2n+1)$ mutants as well as double mutants of the formula $(2n+1+1)$. No modified triploids have as yet been identified, but even if we found them we could not expect to be able to propagate them by seed.

Heterozygous tetraploid plants also show curious ratios, according to whether there are 1, 2, or 3 doses of the dominant factor. Duplex plants give a 35: 1 ratio when selfed and the different types in the offspring segregate in a characteristic fashion.

In the tetraploids we may have a single extra chromosome in one set making a simple $(4n+1)$ mutant, or 2 chromosomes in a set making a simple $(4n+2)$ mutant. We have two cases of a tetraploid with a deficiency in one set, producing a $(4n-1)$ mutant.

Up to the present time, except for Gregory's work on tetraploid *Primulas* (5) which was correctly interpreted by Muller (6), Mendelian research has dealt almost exclusively with disomic inheritance. Our work with the jimsons and the recent investigations of Bridges on triploid *Drosophilas* offer an opportunity for the rather novel study of trisomic, tetrasomic and pentasomic inheritance. We do not believe, however, that the jimson weed is peculiar among plants in giving rise to chromosomal mutants.

The unbalancing effect of the extra chromosomes can best be illustrated by extra chromosomes in the Globe set. The $(2n+2)$ Globe has two extra chromosomes in the Globe set and hence should show a greater divergence from normal than the Globe with only one extra chromosome. Such is the case. The simple $(2n+1)$ Globe (like other mutants of this type) is less vigorous in growth than normals. The $(2n+2)$ Globe is still less vigorous than the more common $(2n+1)$ Globe. From fig. 1 it will be seen, further, that the Globe characters in the $(2n+2)$ Globe on the left, such as broadness of leaves, fatness of bud, and density of foliage, are much further developed

than in the $(2n+1)$ Globe at the right, which has only one extra chromosome.

Photographs of capsules (Fig. 7) will further illustrate the idea of unbalance. Unfortunately the $(2n+2)$ Globe just mentioned fruits poorly and none of its capsules were available when the fruits of the other types were photographed. Later a photograph of a capsule was made to the same scale, and inserted in the proper place in the series. It will be evident that the Globe characters of relative stockiness of spines and depression of capsules are more marked in the $(2n+2)$ Globe where there are 2 extra chromosomes in the Globe set than in the $(2n+1)$ Globe on the left where there is only one extra chromosome in this particular set. Likewise in the modified tetraploids the (plus 2) Globe on the right is more Globe-like than the (plus 1) Globe beside it.

The degree of unbalance of chromosomes in the nuclei may be given a quantitative expression. Thus in the $(2n+1)$ Globe, the extra chromosome produces an excess of one over the balanced $2n$ condition. The nucleus is overbalanced by the active factors in a single Globe chromosome. This unbalance may be said to be 1 over $2n$. In a similar way the $(2n+2)$ Globe with 2 extra chromosomes has an unbalance of 2 over $2n$. Having in mind these quantitative differences one would expect the $(4n+1)$ Globe with an unbalance of 1 over $4n$ to show a less marked expression of the Globe characters than the $(2n+1)$ Globe with an unbalance of 1 over $2n$. They are, in fact, less readily recognized in recording our pedigrees. The relation of unbalance enabled us to predict the possibility of finding $(4n+2)$ Globes with an unbalance of 2 over $4n$ which one would expect to be as distinct in appearance as $(2n+1)$ Globes with an equivalent unbalance of 1 over $2n$. The prediction has been fulfilled and we are led to expect the appearance of Globes with 3, and Globes with 4 extra chromosomes in the Globe set, if tetraploid plants can endure the extreme unbalance of 4 over $4n$, the equivalent of 2 over $2n$ obtained in the $(2n+2)$ Globe.

It must be emphasized that our quantitative expressions of unbalance hold strictly only for the chromosomal numbers in reference to a single set, and not necessarily for the somatic characters conditioned by them, although the nuclear unbalance seems to be reflected in the somatic

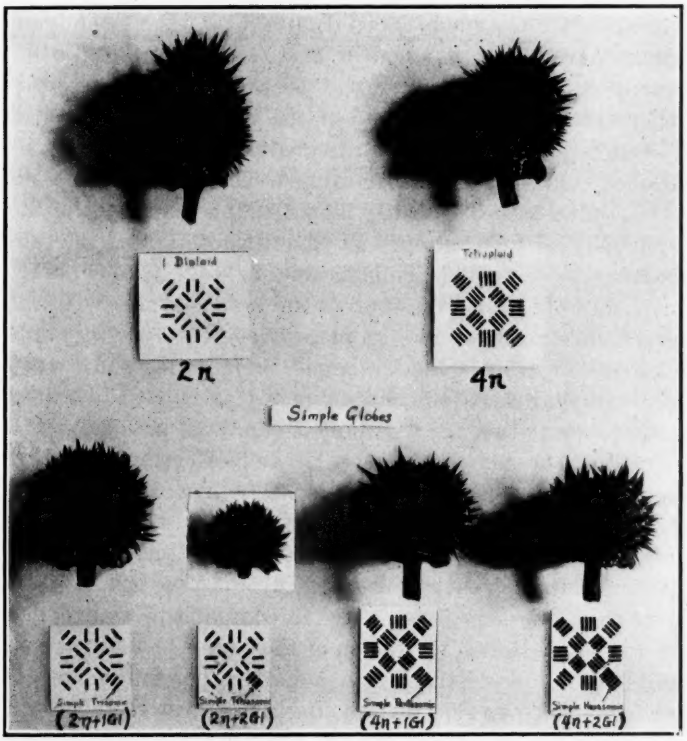

FIG. 7. Above, capsules with diagrams of a diploid ($2n$) and a tetraploid ($4n$). Below, capsules with diagrams of the different Globe mutants.

appearance, at least in the Globe series just discussed. In double mutants, moreover, somatic effects may be intensified or largely neutralized by individual genes in the two extra chromosomes, and an easy expression of the combined unbalance which they exert will therefore be impossible.

The structural characters have been taken for illustra-

tion from a particular part of a single mutant, the Globe. A more detailed study of changes in external and internal morphology brought about by the presence of specific extra chromosomes in the several mutants is being undertaken in cooperation with Dr. Sinnott.

The unbalancing effect of an extra chromosome is shown in the lessened vigor of mutant plants. Thus from Globe parents as an example of $(2n + 1)$ mutants, ordinarily only one quarter of the offspring to reach recordable size are Globes, instead of the 50 per cent. expected. Moreover, when the plants are crowded the proportion of Globes surviving is considerably lessened.

We have been discussing the unbalance as affecting the sporophytic generation. In the gametophyte, the unbalance is doubled. Thus from $(2n + 1)$ Globe plants with an unbalance of 1 over $2n$ the pollen grains with the extra chromosome have an unbalance of 1 over n. This extreme unbalance hinders their functioning and brings it about that the Globe character is transmitted to only a slight extent through the pollen (under 2 per cent. in a considerable series of crosses). It is of interest in this connection to note the results of selfing and crossing Globes of the tetraploid series. The unbalance in a $(4n + 1)$ Globe is 1 over $4n$, while the unbalance in its pollen grains which carry the extra chromosome is 1 over $2n$. Due to this lessened unbalance in comparison with pollen of $(2n + 1)$ Globes, the pollen of the $(4n + 1)$ Globe transmits the Globe character to a higher percentage of its progeny (14 per cent. in the single pedigree tested), and partially for the same reason we have obtained higher proportions of Globes in the offspring from selfing such $(4n + 1)$ Globes (a total of about 60 per cent. in a single experiment). A more specific study of the effect of extra chromosomes upon the gametophyte is being undertaken in cooperation with Dr. Buchholz.

It will not be advisable at the present stage of our investigations to discuss the possible external and internal factors which may induce the chromosomal aberrations which form the basis of our common mutations in *Datura*.

A study of the effects of radium rays undertaken in cooperation with Dr. Gager has given results which, although in an early stage of the experiment, appear suggestive in this connection. Other stimuli are being tested which appear to induce irregularities in the distribution of chromosomes to the pollen grains. It will be a matter of theoretical interest to be able to control experimentally the production of chromosomal mutations. It might also prove to be of considerable economic importance to be able to produce at will the full range of chromosomal mutants in any plants, especially in those which are propagated by vegetative means.

To us, one of the most interesting features of the *Datura* work is the possibility afforded of analyzing the influence of individual chromosomes upon both the morphology and physiology of the plant without waiting for gene mutations. Evidence is at hand which indicates that every chromosome in *Datura* carries factors which influence the expression of the so-called unit character purple pigmentation. Our work so far we believe adds evidence to the conclusion that the mature organism—plant or animal—is not a structure like a child's house of blocks, made up of separate unit characters, nor is it determined by separate and unrelated unit factors. It is rather the resultant of a whole series of interacting and more or less conflicting forces contained in the individual chromosomes.

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VARIATION DUE TO CHANGE IN THE INDIVIDUAL GENE[1]

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I. THE RELATION BETWEEN THE GENES AND THE CHARACTERS OF THE ORGANISM

THE present paper will be concerned rather with problems, and the possible means of attacking them, than with the details of cases and data. The opening up of these new problems is due to the fundamental contribution which genetics has made to cell physiology within the last decade. This contribution, which has so far scarcely been assimilated by the general physiologists themselves, consists in the demonstration that, besides the ordinary proteins, carbohydrates, lipoids, and extractives, of their several types, there are present within the cell *thousands* of distinct substances—the "genes"; these genes exist as ultramicroscopic particles; their influences nevertheless permeate the entire cell, and they play a fundamental rôle in determining the nature of all cell substances, cell structures, and cell activities. Through these cell effects, in turn, the genes affect the entire organism.

It is not mere guesswork to say that the genes are ultra-microscopic bodies. For the work on *Drosophila* has not only proved that the genes are in the chromosomes, in definite positions, but it has shown that there must be hundreds of such genes within each of the larger chromosomes, although the length of these chromosomes is not over a few microns. If, then, we divide the size of the chromosome by the minimum number of its genes, we find that the latter are particles too small to give a visible image.

The chemical composition of the genes, and the formulæ of their reactions, remain as yet quite unknown. We do know, for example, that in certain cases a given

[1] Contribution No. 156.

pair of genes will determine the existence of a particular enzyme (concerned in pigment production), that another pair of genes will determine whether or not a certain agglutinin shall exist in the blood, a third pair will determine whether homogentisic acid is secreted into the urine ("alkaptonuria"), and so forth. But it would be absurd, in the third case, to conclude that on this account the gene itself consists of homogentisic acid, or any related substance, and it would be similarly absurd, therefore, to regard cases of the former kind as giving any evidence that the gene *is* an enzyme, or an agglutinin-like body. The reactions whereby the genes produce their ultimate effects are too complex for such inferences. Each of these effects, which we call a "character" of the organism, is the product of a highly complex, intricate, and delicately balanced system of reactions, caused by the interaction of countless genes, and every organic structure and activity is therefore liable to become increased, diminished, abolished, or altered in some other way, when the balance of the reaction system is disturbed by an alteration in the nature or the relative quantities of any of the component genes of the system. To return now to these genes themselves.

II. The Problem of Gene Mutability

The most distinctive characteristic of each of these ultra-microscopic particles—that characteristic whereby we identify it as a gene—is its property of self-propagation: the fact that, within the complicated environment of the cell protoplasm, it reacts in such a way as to convert some of the common surrounding material into an end-product identical in kind with the original gene itself. This action fulfills the chemist's definition of "autocatalysis"; it is what the physiologist would call "growth"; and when it passes through more than one generation it becomes "heredity." It may be observed that this reaction is in each instance a rather highly localized one, since the new material is laid down by the side of the original gene.

The fact that the genes have this autocatalytic power is in itself sufficiently striking, for they are undoubtedly complex substances, and it is difficult to understand by what strange coincidence of chemistry a gene can happen to have just that very special series of physico-chemical effects upon its surroundings which produces—of all possible end-products—just this particular one, which is identical with its own complex structure. But the most remarkable feature of the situation is not this oft-noted autocatalytic action in itself—it is the fact that, when the structure of the gene becomes changed, through some "chance variation," the catalytic property of the gene may[2] become correspondingly changed, in such a way as to leave it still *auto*catalytic. In other words, the change in gene structure—accidental though it was—has somehow resulted in a change of exactly *appropriate* nature in the catalytic reactions, so that the new reactions are now accurately adapted to produce more material just like that in the new changed gene itself. It is this paradoxical phenomenon which is implied in the expression "variation due to change in the individual gene," or, as it is often called, "mutation."

What sort of structure must the gene possess to permit it to mutate in this way? Since, through change after change in the gene, this same phenomenon persists, it is evident that it must depend upon some general feature of gene construction—common to all genes—which gives each one a *general* autocatalytic power—a "carte blanche"—to build material of whatever specific sort it itself happens to be composed of. This general principle of gene structure might, on the one hand, mean nothing more than the possession by each gene of some very simple character, such as a particular radicle or "side-chain"—alike in them all—which enables each gene to enter into combination with certain highly organized materials in the outer protoplasm, in such a way as to result in the formation, "by" the protoplasm, of more material like this gene which is in combination with it. In

[2] It is of course conceivable, and even unavoidable, that *some* types of changes do destroy the gene's autocatalytic power, and thus result in its eventual loss.

that case the gene itself would only initiate and guide the direction of the reaction. On the other hand, the extreme alternative to such a conception has been generally assumed, perhaps gratuitously, in nearly all previous theories concerning hereditary units; this postulates that the chief feature of the autocatalytic mechanism resides in the structure of the genes themselves, and that the outer protoplasm does little more than provide the building material. In either case, the question as to what the general principle of gene construction is, that permits this phenomenon of mutable autocatalysis, is the most fundamental question of genetics.

The subject of gene variation is an important one, however, not only on account of the apparent problem that is thus inherent in it, but also because this same peculiar phenomenon that it involves lies at the root of organic evolution, and hence of all the vital phenomena which have resulted from evolution. It is commonly said that evolution rests upon two foundations—inheritance and variation; but there is a subtle and important error here. Inheritance by itself leads to no change, and variation leads to no permanent change, unless the variations themselves are heritable. Thus it is not inheritance *and* variation which bring about evolution, but the inheritance *of* variation, and this in turn is due to the general principle of gene construction which causes the persistence of autocatalysis despite the alteration in structure of the gene itself. Given, now, any material or collection of materials having this one unusual characteristic, and evolution would automatically follow, for this material would, after a time, through the accumulation, competition and selective spreading of the self-propagated variations, come to differ from ordinary inorganic matter in innumerable respects, in addition to the original difference in its mode of catalysis. There would thus result a wide gap between this matter and other matter, which would keep growing wider, with the increasing complexity, diversity and so-called "adaptation" of the selected mutable material.

III. A POSSIBLE ATTACK THROUGH CHROMOSOME BEHAVIOR

In thus recognizing the nature and the importance of the problem involved in gene mutability have we now entered into a *cul de sac,* or is there some way of proceeding further so as to get at the physical basis of this peculiar property of the gene? The problems of growth, variation and related processes seemed difficult enough to attack even when we thought of them as inherent in the organism as a whole or the cell as a whole—how now can we get at them when they have been driven back, to some extent at least, within the limits of an invisible particle? A gene can not effectively be ground in a mortar, or distilled in a retort, and although the physico-chemical investigation of other biological substances may conceivably help us, by analogy, to understand its structure, there seems at present no method of approach along this line.

There is, however, another possible method of approach available: that is, to study the behavior of the chromosomes, as influenced by their contained genes, in their various physical reactions of segregation, crossing over, division, synapsis, etc. This may at first sight seem very remote from the problem of getting at the structural principle that allows mutability in the gene, but I am inclined to think that such studies of synaptic attraction between chromosomes may be especially enlightening in this connection, because the most remarkable thing we know about genes—besides their mutable autocatalytic power—is the highly specific attraction which like genes (or local products formed by them) show for each other. As in the case of the autocatalytic forces, so here the attractive forces of the gene are somehow exactly adjusted so as to react in relation to more material of the same complicated kind. Moreover, when the gene mutates, the forces become readjusted, so that they may now attract material of the new kind; this shows that the attractive or synaptic property of the gene, as well as its catalytic property, is not primarily dependent on its specific structure, but on some general principle of its make-up, that

causes whatever specific structure it has to be auto-attractive (and autocatalytic).

This auto-attraction is evidently a strong force, exerting an appreciable effect against the non-specific mutual repulsions of the chromosomes, over measurable microscopic distances much larger than in the case of the ordinary forces of so-called cohesion, adhesion and adsorption known to physical science. In this sense, then, the physicist has no parallel for this force. There seems, however, to be no way of escaping the conclusion that in the last analysis it must be of the same nature as these other forces which cause inorganic substances to have specific attractions for each other, according to their chemical composition. These inorganic forces, according to the newer physics, depend upon the arrangement and mode of motion of the electrons constituting the molecules, which set up electro-magnetic fields of force of specific patterns. To find the principle peculiar to the construction of the force-field pattern of genes would accordingly be requisite for solving the problem of their tremendous auto-attraction.

Now, according to Troland (1917), the growth of crystals from a solution is due to an attraction between the solid crystal and the molecules in solution caused by the similarity of their force field patterns, somewhat as similarly shaped magnets might attract each other—north to south poles—and Troland maintains that essentially the same mechanism must operate in the autocatalysis of the hereditary particles. If he is right, each different portion of the gene structure must—like a crystal—attract to itself from the protoplasm materials of a similar kind, thus moulding next to the original gene another structure with similar parts, identically arranged, which then become bound together to form another gene, a replica of the first. This does not solve the question of what the general principle of gene construction is, which permits it to retain, like a crystal, these properties of auto-attraction,[3] but if the main point is correct, that

[3] It can hardly be true, as Troland intimates, that all similar fields attract each other more than they do dissimilar fields, otherwise all substances would be autocatalytic, and, in fact, no substances would be soluble. More-

the autocatalysis is an expression of specific attractions between portions of the gene and similar protoplasmic building blocks (dependent on their force-field patterns), it is evident that the very same forces which cause the genes to grow should also cause like genes to attract each other, but much more strongly, since here all the individual attractive forces of the different parts of the gene are summated. If the two phenomena are thus really dependent on a common principle in the make-up of the gene, progress made in the study of one of them should help in the solution of the other.

Great opportunities are now open for the study of the nature of the synaptic attraction, especially through the discovery of various races having abnormal numbers of chromosomes. Here we have already the finding by Belling, that where three like chromosomes are present, the close union of any two tends to exclude their close union with the third. This is very suggestive, because the same thing is found in the cases of specific attractions between inorganic particles, that are due to their force field patterns. And through Bridges' finding of triploid *Drosophila,* the attraction phenomena can now be brought down to a definitely genic basis, by the introduction of specific genes—especially those known to influence chromosome behavior—into one of the chromosomes of a triad. The amount of influence of this gene on attraction may then be tested quantitatively, by genetic determination of the frequencies of the various possible types of segregation. By extending such studies to include the effect of various conditions of the environment—such as temperature, electrostatic stresses, etc.—in the presence of the different genetic situations, a considerable field is opened up.

This suggested connection between chromosome behavior and gene structure is as yet, however, only a possibility. It must not be forgotten that at present we can

over, if the parts of a molecule are in any kind of "solid," three dimensional formation, it would seem that those in the middle would scarcely have opportunity to exert the moulding effect above mentioned. It therefore appears that a special manner of construction must be necessary, in order that a complicated structure like a gene may exert such an effect.

not be sure that the synaptic attraction is exerted by the genes themselves rather than by local products of them, and it is also problematical whether the chief part of the mechanism of autocatalysis resides within the genes rather than in the "protoplasm." Meanwhile, the method is worth following up, simply because it is one of our few conceivable modes of approach to an all-important problem.

It may also be recalled in this connection that besides the genes in the chromosomes there is at least one similarly autocatalytic material in the chloroplastids, which likewise may become permanently changed, or else lost, as has been shown by various studies on chlorophyll inheritance. Whether this plastid substance is similar to the genes in the chromosomes we can not say, but of course it can not be seen to show synaptic attraction, and could not be studied by the method suggested above.[4]

IV. The Attack through Studies of Mutation

There is, however, another method of attack, in a sense more direct, and not open to the above criticisms. That is the method of investigating the individual gene, and the structure that permits it to change, through a study of the changes themselves that occur in it, as observed by the test of breeding and development. It was through the investigation of the *changes* in the chromosomes—caused by crossing over—that the structure of the chromosomes was analyzed into their constituent genes in line formation; it was through study of molecular changes that molecules were analyzed into atoms tied together in definite ways, and it has been finally the rather recent finding of changes in atoms and investigation of the resulting pieces, that has led us to the present analysis of atomic structure into positive and negative electrons having characteristic arrangements. Similarly, to understand the properties and possibilities of the individual gene, we must study the mutations as directly as possible, and bring the results to bear upon our problem.

[4] It may be that there are still other elements in the cell which have the nature of genes, but as no critical evidence has ever been adduced for their existence, it would be highly hazardous to postulate them.

(*a*) *The Quality and Quantity of the Change*

In spite of the fact that the drawing of inferences concerning the gene is very much hindered, in this method, on account of the remoteness of the gene-cause from its character-effect, one salient point stands out already. It is that the change is not always a mere loss of material, because clear-cut reverse mutations have been obtained in corn, *Drosophila, Portulaca,* and probably elsewhere. If the original mutation was a loss, the reverse must be a gain. Secondly, the mutations in many cases seem not to be quantitative at all, since the different allelomorphs formed by mutations of one original gene often fail to form a single linear series. One case, in fact, is known in which the allelomorphs even affect totally different characters: this is the case of the truncate series, in which I have found that different mutant genes at the same locus may cause either a shortening of the wing, an eruption on the thorax, a lethal effect, or any combination of two or three of these characters. In such a case we may be dealing either with changes of different types occurring in the same material or with changes (possibly quantitative changes, similar in type) occurring in different component parts of one gene. Owing to the universal applicability of the latter interpretation, even where allelomorphs do not form a linear series, it can not be categorically denied, in any individual case, that the changes may be merely quantitative changes of some *part* of the gene. If all changes were thus quantitative, even in this limited sense of a loss or gain of part of the gene, our problem of why the changed gene still seems to be autocatalytic would in the main disappear, but such a situation is excluded a priori since in that case the thousands of genes now existing could never have evolved.

Although a given gene may thus change in various ways, it is important to note that there is a strong tendency for any given gene to have its changes of a particular kind, and to mutate in one direction rather than in another. And although mutation certainly does not always consist of loss, it often gives effects that might be termed losses. In the case of the mutant genes for

bent and eyeless in the fourth chromosome of *Drosophila* it has even been proved, by Bridges, that the effects are of exactly the same kind, although of lesser intensity, than those produced by the entire loss of the chromosome in which they lie, for flies having bent or eyeless in one chromosome and lacking the homologous chromosome are even more bent, or more eyeless, than those having a homologous chromosome that also contains the gene in question. The fact that mutations are usually recessive might be taken as pointing in the same direction, since it has been found in several cases that the loss of genes—as evidenced by the absence of an entire chromosome of one pair—tends to be much more nearly recessive than dominant in its effect.

The effect of mutations in causing a loss in the characters of the organism should, however, be sharply distinguished from the question of whether the gene has undergone any loss. It is generally true that mutations are much more apt to cause an apparent loss in character than a gain, but the obvious explanation for that is, not because the gene tends to lose something, but because most characters require for proper development a nicely adjusted train of processes, and so any change in the genes—no matter whether loss, gain, substitution or rearrangement—is more likely to throw the developmental mechanism out of gear, and give a "weaker" result, than to intensify it. For this reason, too, the most frequent kind of mutation of all is the lethal, which leads to the loss of the entire organism, but we do not conclude from this that all the genes had been lost at the time of the mutation. The explanation for this tendency for most changes to be degenerative, and also for the fact that certain other kinds of changes—like that from red to pink eye in *Drosophila*—are more frequent than others—such as red to brown or green eye—lies rather in developmental mechanics than in genetics. It is because the developmental processes are more unstable in one direction than another, and easier to push "downhill" than up, and so any mutations that occur—no matter what the gene change is like—are more apt to have these *effects*

than the other *effects*. If now selection is removed in regard to any particular character, these character changes which occur more readily must accumulate, giving apparent orthogenesis, disappearance of unused organs, of unused physiological capabilities, and so forth. As we shall see later, however, the changes are not so frequent or numerous that they could ordinarily push evolution in such a direction against selection and against the immediate interests of the organism.

In regard to the magnitude of the somatic effect produced by the gene variation, the *Drosophila* results show that there the smaller character changes occur oftener than large ones. The reason for this is again probably to be found in developmental mechanics, owing to the fact that there are usually more genes slightly affecting a given character than those playing an essential rôle in its formation. The evidence proves that there are still more genes whose change does not affect the given character at all—no matter what this character may be, unless it is life itself—and this raises the question as to how many mutations are absolutely unnoticed, affecting no character, or no detectable character, to any appreciable extent at all. Certainly there must be many such mutations, judging by the frequency with which "modifying factors" arise, which produce an effect only in the presence of a special genetic complex not ordinarily present.

(*b*) *The Localization of the Change*

Certain evidence concerning the causation of mutations has also been obtained by studying the relations of their occurrence to one another. Hitherto it has nearly always been found that only one mutation has occurred at a time, restricted to a single gene in the cell. I must omit from consideration here the two interesting cases of deficiency, found by Bridges and by Mohr, in each of which it seems certain that an entire region of a chromosome, with its whole cargo of genes, changed or was lost, and also a certain peculiar case, not yet cleared up, which has recently been reported by Nilson-Ehle; these important

cases stand alone. Aside from them, there are only two instances in which two (or more) new mutant genes have been proved to have been present in the same gamete. Both of these are cases in *Drosophila*—reported by Muller and Altenburg (1921)—in which a gamete contained two new sex-linked lethals; two cases are not a greater number than was to have been expected from a random distribution of mutations, judging by the frequency with which single mutant lethals were found in the same experiments. Ordinarily, then, the event that causes the mutation is specific, affecting just one particular kind of gene of all the thousands present in the cell. That this specificity is due to a spatial limitation rather than a chemical one is shown by the fact that when the single gene changes the other one, of identical composition, located near by in the homologous chromosome of the same cell, remains unaffected. This has been proved by Emerson in corn, by Blakeslee in *Portulaca,* and I have shown there is strong evidence for it in *Drosophila.* Hence these mutations are not caused by some general pervasive influence, but are due to " accidents " occurring on a molecular scale. When the molecular or atomic motions chance to take a particular form, to which the gene is vulnerable, then the mutation occurs.

It will even be possible to determine whether the entire gene changes at once, or whether the gene consists of several molecules or particles, one of which may change at a time. This point can be settled in organisms having determinate cleavage, by studies of the distribution of the mutant character in somatically mosaic mutants. If there is a group of particles in the gene, then when one particle changes it will be distributed irregularly among the descendant cells, owing to the random orientation of the two halves of the chromosome on the mitotic spindles of succeeding divisions,[5] but if there is only one particle to

[5] This depends on the assumption that if the gene does consist of several particles, the halves of the chromosomes, at each division, receive a random sample of these particles. That is almost a necessary assumption, since a gene formed of particles each one of which was separately partitioned at division would tend not to persist as such, for the occurrence of mutation in one particle after the other would in time differentiate the gene into a number of different genes consisting of one particle each.

change, its mutation must affect all of the cells in a *bloc,* that are descended from the mutant cell.

(*c*) *The Conditions under which the Change occurs*

But the method that appears to have most scope and promise is the experimental one of investigating the conditions under which mutations occur. This requires studies of mutation frequency under various methods of handling the organisms. As yet, extremely little has been done along this line. That is because, in the past, a mutation was considered a windfall, and the expression "mutation frequency" would have seemed a contradiction in terms. To attempt to study it would have seemed as absurd as to study the conditions affecting the distribution of dollar bills on the sidewalk. You were simply fortunate if you found one. Not even controls, giving the "normal" rate of mutation—if indeed there is such a thing—were attempted.[6] Of late, however, we may say that certain very exceptional banking houses have been found, in front of which the dollars fall more frequently—in other words, specially mutable genes have been discovered, that are beginning to yield abundant data at the hands of Nilsson-Ehle, Zeleny, Emerson, Anderson and others. For some of these mutable genes the rate of change is found to be so rapid that at the end of a few decades half of the genes descended from those originally present would have become changed. After these genes have once mutated, however, their previous mutability no longer holds. In addition to this "banking house method" there are also methods, employed by Altenburg and myself, for—as it were—automatically sweeping up wide areas of the streets and sifting the collections for the valuables. By these special genetic methods of reaping mutations we have recently shown that the ordinary genes of *Drosophila*—unlike the mutable genes above—would usually require at least a thousand years—prob-

[6] Studies of "mutation frequency" had of course been made in the Œnotheras, but as we now know that these were not studies of the rate of gene change but of the frequencies of crossing over and of chromosome aberrations they may be neglected for our present purposes.

ably very much more—before half of them became changed. This puts their stability about on a par with, if not much higher than, that of atoms of radium—to use a fairly familiar analogy. Since, even in these latter experiments, many of the mutations probably occurred within a relatively few rather highly mutable genes, it is likely that most of the genes have a stability far higher than this result suggests.

The above mutation rates are mere first gleanings—we have yet to find how different conditions affect the occurrence of mutations. There had so far been only the negative findings that mutation is not confined to one sex (Muller and Altenburg, 1919; Zeleny, 1921), or to any one stage in the life cycle (Bridges, 1919; Muller, 1920; Zeleny, 1921), Zeleny's finding that bar-mutation is not influenced by recency of origin of the gene (1921), and the as yet inconclusive differences found by Altenburg and myself for mutation rate at different temperatures (1919), until at this year's meeting of the botanists Emerson announced the definite discovery of the influence of a genetic factor in corn upon the mutation rate in its allelomorph, and Anderson the finding of an influence upon mutation in this same gene, caused by developmental conditions—the mutations from white to red of the mutable gene studied occurring far more frequently in the cells of the more mature ear than in those of the younger ear. These two results at least tell us decisively that mutation is not a sacred, inviolable, unapproachable process: it may be altered. These are the first steps; the way now lies open broad for exploration.

It is true that I have left out of account here the reported findings by several investigators, of genetic variations caused by treatments with various toxic substances and with certain other unusual conditions. In most of these cases, however, the claim has not been made that actual gene changes have been caused: the results have usually not been analyzed genetically and were in fact not analyzable genetically; they could just as well be interpreted to be due to abnormalities in the distribution of genes—for instance, chromosome abnormalities like

those which Mavor has recently produced with X-rays—as to be due to actual gene mutations. But even if they were due to real genic differences, the possibility has in most cases by no means been excluded (1) that these genic differences were present in the stock to begin with, and merely became sorted out unequally, through random segregation; or (2) that other, invisible genic differences were present which, after random sorting out, themselves caused differences in mutation rate between the different lines. Certain recent results by Altenburg and myself suggest that genic differences, affecting mutation rate, may be not uncommon. To guard against either of these possibilities it would have been necessary to test the stocks out by a thorough course of inbreeding beforehand, or else to have run at least half a dozen different pairs of parallel lines of the control and treated series, and to have obtained a definite difference in the same direction between the two lines of *each* pair; otherwise it can be proved by the theory of "probable error" that the differences observed may have been a mere matter of random sampling among genic differences originally present. Accumulating large numbers of abnormal or inferior individuals by selective propagation of one or two of the treated lines—as has been done in some cases—adds nothing to the significance of the results.

At best, however, these genetically unrefined methods would be quite insensitive to mutations occurring at anything like ordinary frequency, or to such differences in mutation rate as have already been found in the analytical experiments on mutation frequency. And it seems quite possible that larger differences than these will not easily be hit upon, at least not in the early stages of our investigations, in view of the evidence that mutation is ordinarily due to an accident on an ultramicroscopic scale, rather than directly caused by influences pervading the organism. For the present, then, it appears most promising to employ organisms in which the genetic composition can be controlled and analyzed, and to use genetic methods that are sensitive enough to disclose mutations occurring in the control as well as in the treated individ-

uals. In this way relatively slight variations in mutation frequency, caused by the special treatments, can be determined, and from the conditions found to alter the mutation rate slightly we might finally work up to those which affect it most markedly. The only methods now meeting this requirement are those in which a particular mutable gene is followed, and those in which many homozygous or else genetically controlled lines can be run in parallel, either by parthenogenesis, self-fertilization, balanced lethals or other special genetic means, and later analyzed, through sexual reproduction, segregation and crossing over.

V. Other Possibilities

We can not, however, set fixed limits to the possibilities of research. We should not wish to deny that some new and unusual method may at any time be found of directly producing mutations. For example, the phenomena now being worked out by Guyer may be a case in point. There is a curious analogy between the reactions of immunity and the phenomena of heredity, in apparently fundamental respects,[7] and any results that seem to connect the two are worth following to the limit.

Finally, there is a phenomenon related to immunity, of still more striking nature, which must not be neglected by geneticists. This is the d'Hérelle phenomenon. D'Hérelle found in 1917 that the presence of dysentery bacilli in the body caused the production there of a filterable substance, emitted in the stools, which had a lethal and in fact dissolving action on the corresponding type of bacteria, if a drop of it were applied to a colony of the bacteria that were under cultivation. So far, there would be nothing to distinguish this phenomenon from im-

[7] I refer here to the remarkable specificity with which a particular complex antigen calls forth processes that construct for it an antibody that is attracted to it and fits it "like lock and key," followed by further processes that cause more and more of the antibody to be reproduced. *If* the antigen were a gene, which could be slightly altered by the cell to form the antibody that neutralized it—as some enzymes can be slightly changed by heating so that they counteract the previous active enzyme—and if this antibody-gene then became implanted in the cell so as to keep on growing, all the phenomena of immunity would be produced.

munity. But he further found that when a drop of the affected colony was applied to a second living colony, the second colony would be killed; a drop from the second would kill a third colony, and so on indefinitely. In other words, the substance, when applied to colonies of bacteria, became multiplied or increased, and could be so increased indefinitely; it was self-propagable. It fulfills, then, the definition of an autocatalytic substance, and although it may really be of very different composition and work by a totally different mechanism from the genes in the chromosomes, it also fulfills our definition of a gene.[8] But the resemblance goes further—it has been found by Gratia that the substance may, through appropriate treatments on other bacteria, become changed (so as to produce a somewhat different effect than before, and attack different bacteria) and still retain its self-propagable nature.

That two distinct kinds of substances—the d'Hérelle substances and the genes—should both possess this most remarkable property of heritable variation or "mutability," each working by a totally different mechanism, is quite conceivable, considering the complexity of protoplasm, yet it would seem a curious coincidence indeed. It would open up the possibility of two totally different kinds of life, working by different mechanisms. On the other hand, if these d'Hérelle bodies were really genes, fundamentally like our chromosome genes, they would give us an utterly new angle from which to attack the gene problem. They are filterable, to some extent isolable, can be handled in test-tubes, and their properties, as shown by their effects on the bacteria, can then be studied after treatment. It would be very rash to call these bodies genes, and yet at present we must confess that there is no distinction known between the genes and them. Hence we can not categorically deny that perhaps we may be able to grind genes in a mortar and cook them in a beaker after all. Must we geneticists become bac-

[8] D'Hérelle himself thought that the substance was a filterable virus parasitic on the bacterium, called forth by the host body. It has since been found that various bacteria each cause the production of D'Hérelle substances which are to some extent specific for the respective bacteria.

teriologists, physiological chemists and physicists, simultaneously with being zoologists and botanists? Let us hope so.

I have purposely tried to paint things in the rosiest possible colors. Actually, the work on the individual gene, and its mutation, is beset with tremendous difficulty. Such progress in it as has been made has been by minute steps and at the cost of infinite labor. Where results are thus meager, all thinking becomes almost equivalent to speculation. But we can not give up thinking on that account, and thereby give up the intellectual incentive to our work. In fact, a wide, unhampered treatment of all possibilities is, in such cases, all the more imperative, in order that we may direct these labors of ours where they have most chance to count. We must provide eyes for action.

The real trouble comes when speculation masquerades as empirical fact. For those who cry out most loudly against " theories " and " hypotheses "—whether these latter be the chromosome theory, the factorial " hypothesis," the theory of crossing over, or any other—are often the very ones most guilty of stating their results in terms that make illegitimate *implicit* assumptions, which they themselves are scarcely aware of simply because they are opposed to dragging " speculation " into the open. Thus they may be finally led into the worst blunders of all. Let us, then, frankly admit the uncertainty of many of the possibilities we have dealt with, using them as a spur to the real work.

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THE ORIGIN OF VARIATIONS IN SEXUAL AND SEX-LIMITED CHARACTERS

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IN dealing with sex and its determination, attention has been most sharply focused upon forms with separate sexes and upon the visible differences between the chromosome groups of the two sexes. The result has been that the formulation of sex-determination has remained in terms of chromosomes, while the modern unit of determination is the gene; and also the subject of sex has been rather separated off from the main body of heredity. My discussion will be largely a process of resolving chromosomes into component genes, and showing that the conception of the nature and action of genes as gained from the study of non-sexual characters is valid in interpreting sex phenomena.

The facts of mutation and of linkage have given us the conception of a gene as a distinct chemical entity having a definite location in a particular chromosome. Each gene is essentially a factory, which is manufacturing a characteristic set of chemical products that are delivered to the common cytoplasm, and that produce development through interaction with each other and with materials from outside. But since the chemicals produced by the different genes are different, some genes will have much effect upon one character and little effect upon another, so that a relatively small proportion of the genes will be actively concerned in producing any given character. Some of these genes tend to make the character more pronounced, and others tend to make it less pronounced, so that the grade of development actually realized by each particular character will be determined by the equilibrium between its modifying genes. The forms into which a given character can be modified are in general quite diverse, but for the sake of simplicity we may call them all plus or minus modifications. If the effectiveness of a given plus or minus modifier is changed by mutation, the grade of the character will shift correspondingly.

We can conceive of the evolution of the sexual

characters of hermaphrodites in terms of successive simple mutations in genes. But to interpret male and female forms with observed differences in number or size of chromosomes and with sex-linked inheritance requires comparison with mutations in which the unit of change is a whole chromosome or section of chromosome instead of a single gene. Such mutations can be understood in terms of the action of component genes as follows. Linkage experiments show that the various kinds of genes are distributed pretty much at random among the various chromosomes and along each chromosome. But since the number of genes with a given tendency is relatively small, any particular small section of chromosome might not contain these genes in the same proportion as they exist in the entire complement, and still less would the normal proportion of every kind be present. The loss of a section of chromosome (a condition known as deficiency) would ordinarily remove more minus than plus modifiers (or vice-versa), and since in that case more plus than minus modifiers would remain in action, the grade of the corresponding character would be shifted in a minus direction. This is the interpretation of the fact that a deficiency may cause many character changes, the complex of altered characters being inherited as a dominant. When a whole chromosome is lost through nondisjunction, the effects are similar to those in deficiency for a section except that they are greater in degree.

The way in which genes act together in producing a character, and the relation of the balance of plus and minus modifiers to deficiency or to the absence of a chromosome may perhaps be made clearer by an analogy. Let us suppose that a man is an ardent stamp collector, and has accumulated a lot of stamps. These stamps are to represent genes, so their number may be put at 5,000 to correspond roughly to the number of genes in *Drosophila*. Among the Russian stamps, especially those of recent issue, there is a very large number of reds, but also a fair number of pinks, and even a few whites. These differences in tint correspond to the plus and minus modifiers of a certain character, namely, the redness of Russian stamps. Now the stamps of different tints are in some definite ratio, whatever that ratio is, and we will call it the

normal ratio or balance. This stamp collector carries his collection around with him, and it fills two big, coat pockets, a trousers pocket, and there are even a few in his vest pocket. But unlike most collectors this one has never taken the trouble to sort over more than a few of his stamps. Meanwhile he strings them together pretty much hit or miss. This stringing stamps together is rather disapproved of by some other stamp collectors, who think that is no way to treat stamps, and each of whom has his own favorite method of arranging them. Because of this hit or miss method of making the strings of stamps the ratio among the different grades of redness of Russian stamps is different in different parts of the strings, and so if some other ardent collector should snip off a piece of one of the strings and carry it away, the remainder of the Russian stamps might have a considerably redder tone, while at the same time the Polish stamps might become bluer. If a whole string were lost, then many of the sets of stamps might have quite different complexions.

Now I have been recently studying the effects of the loss of one of the chromosomes of *Drosophila*,[1] namely, the small round fourth-chromosome, and the phenomena offer striking parallels to those of diœcious sex, including sex-linked inheritance and sex-limited characters. Individuals having only one fourth-chromosome show a change in many characters, among which may be mentioned smaller size, smaller bristles, later hatching, poorer viability, paler body-color, darker trident pattern, shorter blunter wings, etc. Each of these differences corresponds to a character for which the fourth-chromosome was internally unbalanced, that is, for which the ratio of plus to minus modifiers was different from that of the whole group. For all of the characters in which there was an internal preponderance of plus modifiers the grade will be shifted in a minus direction by the loss of the fourth-chromosome, for example, the shorter wings and paler body-color. Likewise the characters that shift in a plus direction, as the darker trident pattern and the large eyes, are characters for which the fourth-chromosome possesses an excess of minus modifiers. In the

[1] *Proc. Nat'l Acad. of Sci.*, 7: 186–192.

male of *Drosophila* there is only one X-chromosome, though there is present a Y-chromosome that can be disregarded, since the evidence from non-disjunction of the X-chromosome shows that it has very little effect upon sex or characters. These individuals with only one X-

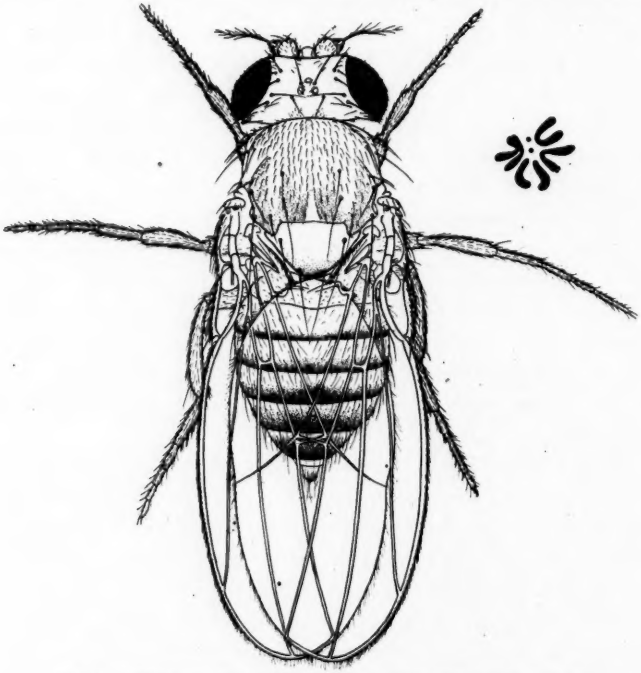

FIG. 1. Wild-type (2n) female, with normal chromosome group.

chromosome likewise show a complex of characters that are different from those shown by the individuals with the normal two X-chromosomes. Among these characters are gonads and genitalia of a type that we call male. The haplo-X individual is also smaller, has smaller bristles, is less viable, hatches later, and differs in other details from the 2-X type that we call female. Each of these differences likewise corresponds to a character for which the balance of the genes in the X is different from that in the group as a whole. The absence of one X leaves in action an unbalanced set of genes which produces male characters. The X-chromosome is a chromosome that is internally unbalanced by an excess of genes that we may call female-producing.

In an outcross of a haplo-IV individual to a normal, the entire complex of characters is inherited as a simple dominant and gives a 1 : 1 ratio, except that the haplo-IV's are less viable. Likewise in outcrosses of haplo-X individuals the entire complex of male characters is in-

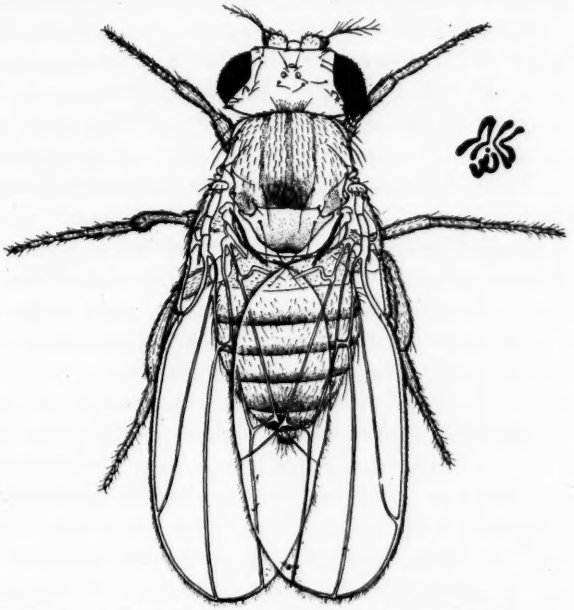

FIG. 2. Haplo-IV (2n–1 IV) female, with chromosome group.

herited as a simple dominant and gives a 1 : 1 ratio except that the haplo-X's are somewhat less viable.

When a haplo-IV individual is mated to a recessive whose gene is in the fourth-chromosome, all the haplo-fourth offspring show this recessive—a behavior that is strictly parallel to sex-linked inheritance; for if a haplo-X individual, that is, a male, is mated to a recessive whose gene is in the X, all the haplo-X offspring show this recessive.

The fourth-chromosome recessive characters present in haplo-IV individuals from the cross of a haplo-fourth to the recessive show a grade of development that is different from their grade as homozygous characters in diplo-IV's. This phenomenon is known as "exaggeration," and is interpreted as the effect of an unbalance within the

normal fourth-chromosome. With respect to a character that is exaggerated in a plus direction the fourth-chromosome has an unbalance in the minus direction. But since the whole complement is in balance, this unbalance within the fourth-chromosome is neutralized by a reciprocal unbalance in the other chromosomes. So the removal of one fourth-chromosome with its excess of minus modifiers leaves the remainder of the genes with an excess of plus modifiers, and these plus modifiers are free to work in the same direction as the recessive gene that is present, and thus to give an even greater effect than the homozygous recesive. Corresponding to these exaggerated fourth-chromosome characters there is a class of sex-linked characters that are exaggerated in the absence of one X-chromosome. These mutant characters show a different grade of development in the male from that which they show in the female. A good example is the race called eosin, in which the male has a much paler eye-color than the eosin female. These characters exaggerated by the absence of an X are called sex-limited. Some of them, like eosin, are exaggerated in a plus direction, corresponding to an excess of minus modifiers within the X-chromosome, while others, such as bobbed, are exaggerated in a minus direction. Thus bobbed, which shows scarcely at all in the males, corresponds to an excess of genes within the X tending to make bristles short, and two X-chromosomes can outweigh the genes in the autosomes that tend to make the bristles long, but one X is not enough to do so.

When haploidy for the fourth-chromosome is combined with mutants whose genes are outside the fourth-chromosome there is of course no effect corresponding to sex-linkage, but there is "exaggeration." Thus, haploidy for the fourth-chromosome exaggerates the third-chromosome mutant Hairless in a plus direction. This type of exaggeration finds its parallel in the 20 or so sex-limited mutations that are not sex-linked. These are mutations whose differential genes are in the autosomes and not in the X and which nevertheless show a different grade of development in the male from that in the female. In these cases also the modifiers of each character are of different weights in the X from the general collection,

and absence of one X leaves a surplus of genes that work in the same or in the opposite direction from that of the mutant in question.

Thus, by studying three kinds of effects, first, the character complexes that result directly, secondly, the exaggerations of the mutant characters whose genes are in the same section or chromosome as that involved in the loss, and thirdly the exaggerations of mutant characters whose genes are in other regions, we can analyse roughly the kinds and the signs of the genes that are in the region in question.

Since sexual and sex-limited characters are shown to rest on the same genetic basis, namely, a preponderance within the X of the plus or the minus modifiers of those characters, it may be questioned whether there is any real difference between these two categories. If the race of the mutant eosin were to become established in nature, a systematist would certainly include this difference in eye-color among his sexual differences. I am of the opinion that there is no difference between these two categories except that we call those sexual that are most closely connected with reproduction.

There is one striking difference between haploidy for X and haploidy for an autosome—namely, that the changes connected with haploidy for autosomes are relatively more numerous and extreme. Haploidy for the second or third autosomes probably produces changes so great as to be lethal, while haploidy for the very small fourth-chromosome produces changes comparable in extent to all those of the male aside from the reproductive organs. The proportion of sex-limited mutant characters is only about a tenth of the total, while X contains about a quarter of the genes. Since the changes in character produced by absence of an X are relatively small, the internal balance of the X must be relatively high. For a high proportion of the characters of the animal, the plus and minus modifiers in the X must be in about the same ratio as in the group as a whole.

The comparison just made between the effects of haploidy for an autosome and the effects normally present in dicœcious sex shows that they have similar genic bases—namely, each is due to differences in the ratio between two

aggregates of genes; and that the X produces its characteristic effects because it contains a preponderance of genes tending to produce the characters that we call female. This point of view receives even stronger and more direct support from a study of cases in which the

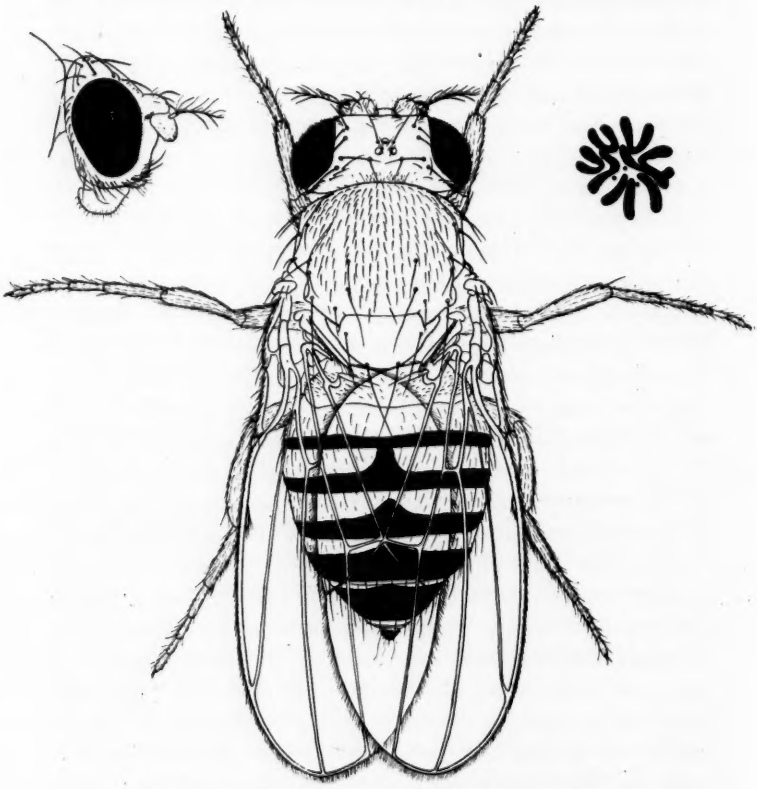

FIG. 3. Triploid (3n) female, with chromosome group.

ratio of X-chromosome to autosomes has been changed, and in which new sex relations are present. These new types of chromosome combinations and of sex take their origin in the occurrence of triploidy in *Drosophila,* for which there is full genetical and cytological proof.[2] The first point is that individuals having three full sets of chromosomes (3n) are females not to be distinguished from normal females except for slight differences in size and proportion that may well be due simply to the greater

[2] *Science,* N. S., **54**: 252–254.

amount of chromatin. The nearly complete identity between the triploid and diploid forms both as to sex and as to non-sexual characters is a splendid evidence that these characters owe their grade to the ratios among the genes, for those ratios are identical in the 3n and 2n forms.

Among the offspring of triploid females are individuals that are neither males nor females but are sex-intermediates, or rather, are mixtures of male and female characters, very similar in type to the intersexes of *Lymantria.*[3] Genetical and cytological proof was obtained that these intersexes in *Drosophila* possess two X-chromosomes and three sets of autosomes. The old formulation of 2X equals ♀ is at once seen to be inadequate, for here we have individuals that have two X-chromosomes and yet are not females. They are shifted out of the female class by the presence of an extra set of autosomes, and thereby the autosomes are proved to play a positive rôle in the production of sex. Since the intersexes differ from females by the assumption of certain male characters this effect of the autosomes is due to an internal preponderance of "male-tendency" genes.

We may now formulate the sex-relations as follows: both sexes are due to the simultaneous action of two opposed sets of genes, one set tending to produce the characters called female and the other to produce the characters called male. These two sets of genes are not equally effective, for in the complement as a whole the female-tendency genes outweigh the male-tendency genes and the diploid (or triploid) form is a female. When the relative number of the female-tendency genes is lowered by the absence of one X, the male-tendency genes outweigh the female and the result is the normal haplo-X male. When the two sets of genes are acting in a ratio between these two extremes, as is the case in the ratio of *2X: 3 sets autosomes,* the result is a sex intermediate—the intersex.

The intersexes as a class can always be easily distinguished from normal males and females by reason of their large size, large coarse-textured eyes and by certain other characters such as scalloped wing-margins. Some of these characters are probably non-sexual effects of the

[3] R. Goldschmidt, *Zeit. f. ind. Abst. u. Vererb.,* 23: 1–199.

triploidy for the autosomes, others are sex-limited. Within the class of intersexes there is a very wide range of fluctuation, on the one hand to flies that are nearly female and on the other to flies that are entirely male in appearance. In an intersex of a given grade the several

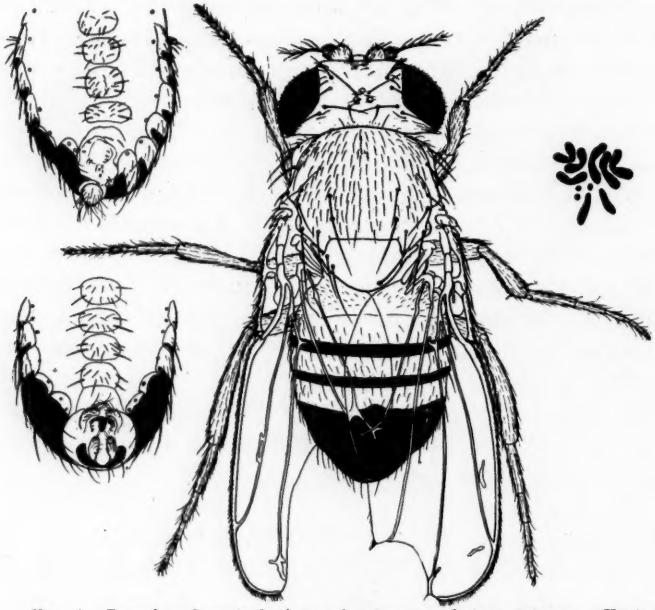

FIG. 4. Dorsal and ventral views of extreme male-type intersex. Ventral view of mid-grade intersex. Chromosome group of intersex showing 2X and 3 sets autosomes.

characters do not all present the same intermediate step between male and female, but, apparently just as in the intersexes of *Lymantria,* some characters are completely male, some completely female, while others are complex mixtures of male and female parts. When the intersexes are classified according to a system of grades, they are seen to be a bimodal class consisting of more "female-type" and more "male-type" intersexes, both of which fluctuate widely and overlap considerably.

The cytological investigation of the intersexes had shown that there are four sub-types of intersexes that differ in the presence or absence of a Y and in having three or only two fourth-chromosomes. It is possible, and there is some slight cytological and genetical evidence in support, that the male- and female-types of

intersexes correspond to the presence of three or of two fourth-chromosomes respectively.

There is another connection in which the wide fluctuations of the intersexes are interesting, namely, the action

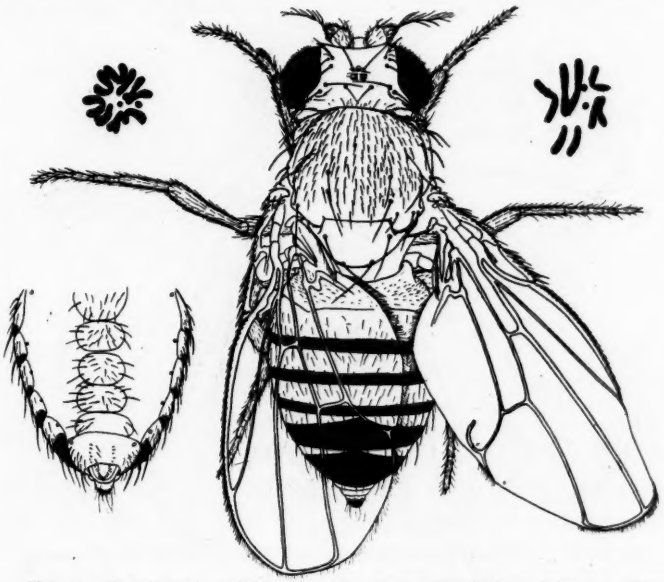

FIG. 5. Dorsal and ventral views of extreme female-type intersex. Two chromosome groups, the left with two IV-chromosomes and a Y, the right with two IV's but no Y.

of environmental factors. The slight range of fluctuation in such a character as miniature-wings in *Drosophila* probably means that there is a critical balance or ratio of plus to minus modifiers beyond which all balances give miniature, at least until the overbalance proceeds so far that a new critical ratio is passed and a new super-miniature character is realized. The balance in miniature is so far beyond the critical balance that only rarely are the environmental factors strong enough to outweigh this overbalance and thus cause fluctuation. In mutants in which the overbalance is slight, there will be both wide fluctuation due to environmental interference and a high susceptibility to modification by other genes, as is notoriously the case with Beaded and with Truncate.

In normal males and females there are high overbalances beyond the critical points, and consequently only slight genetical or fluctuating variations. But in the in-

tersexes these two overbalances in opposite directions cancel each other, and since the two sets of genes are now of almost exactly the same weight the point of balance is between the two critical balances. Accordingly the char-

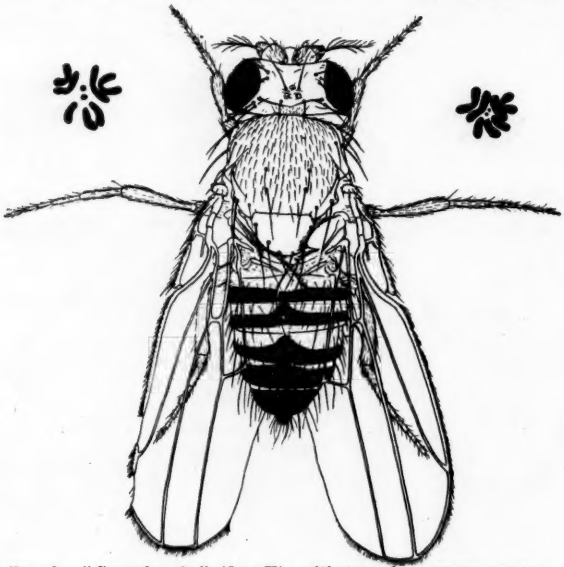

FIG. 6. "Superfemale" (2n + X), with two chromosome groups.

acters of the intersex fluctuate widely with slight environmental differences, and fall into two modes corresponding to the slight difference in balance between two and three of the tiny fourth-chromosomes.

RELATION OF SEX TO CHROMOSOMES IN *Drosophila melanogaster*

Sex		X-chromosomes	Sets Autosomes	Sex Index
Superfemale		3	2	1.5
Female	triploid	3	3	1
	diploid	2	2	1
Intersex	♀-type	2	3(—IV)	.67+
	♂-type	2	3	.67
Male		1	2	.5
Supermale		1	3	.33

The phenomenon of intersexuality might be expected to have a reciprocal phase—namely, supersexes. If the

intersexes result from an intermediate ratio of X to autosomes because the X has a net female tendency, then it might be expected that by increasing the ratio of X to autosomes a superfemale would be produced, and conversely, a supermale by increasing the relative number of

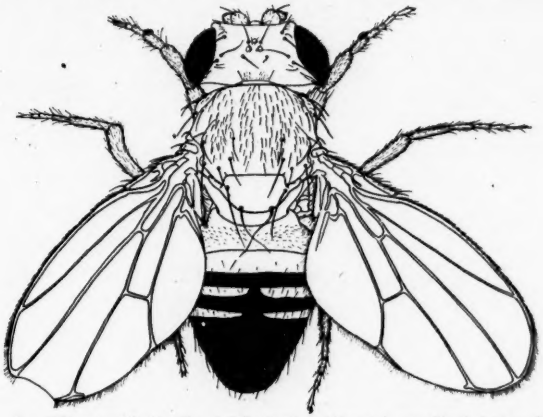

FIG. 7. "Supermale." No cytological evidence; genetical tests show 1X and 3 sets autosomes.

autosomes. Diploid individuals with an extra X-chromosome (2n plus X) have now been identified among the progeny of certain strains of high non-disjunction, among the offspring of triploid females and elsewhere. These flies resemble females but are very inviable and form a distinct character type. They are sterile and sections of the gonads show abnormal ovaries. These differences all result from the unbalance within the X, and are therefore of the sexual-sex-limited category. That these differences are not greater is partly due to the same high internal balance of the X that we met with in analysing males and intersexes, and is partly to be explained on the ground that for many of the characters the overbalance is not yet great enough to pass a second critical point.

Conversely, individuals with one X-chromosome and an extra set of autosomes have been identified among the offspring of triploid females. These are males distinctly different from normal males and sterile.

If there were time, it would be interesting to supplement and modify the view just presented by comparisons with the rich materials elsewhere, and perhaps to speculate as to how this machinery was evolved, and how the genes involved come to expression physiologically.

THE NATURE OF BUD VARIATIONS AS INDICATED BY THEIR MODE OF INHERITANCE[1]

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THE title limits this account to such bud variations as have been studied critically with respect to their inheritance in sexual reproduction. The further limitation of time makes it necessary that I choose from among such studies certain cases to serve as illustrations of the several types of bud variation. I shall, therefore, attempt no complete review of the researches bearing on the problem at hand.

A survey of published accounts of bud-variation studies shows that as yet comparatively little is definitely known of the real nature of these vegetative sports. It seems not unlikely, however, that to point out some of the problems suggested by these studies and, where possible, to note modes of attack may serve the purpose of this symposium quite as well as a rehearsal of known facts and their interpretation.

As here used, the term bud variation is synonymous with vegetative as contrasted with seminal variation. The term somatic variation may also be employed to the same effect, provided it is not thereby intended to exclude cases in which the germ tract as well as the soma is involved. At the outset, however, there must be imposed on any of these terms, for the purpose of this discussion at least, the limitation that the variation involves a change in the genetic constitution of the parts affected.

The expressions somatic mutation and somatic segregation are specific terms and as such are not to be used interchangeably with the more general terms somatic, vegetative, or bud variations. Moreover, to speak of a particular vegetative variation as a case of somatic muta-

[1] Paper No. 94, Department of Plant Breeding, Cornell University, Ithaca, New York.

tion or of somatic segregation without basis from critical inheritance or cytological studies is to prejudge the nature of the observed modification.

Frequency of Somatic Variations

Attempts have been made to estimate the relative frequency of vegetative and seminal variations in plants, but little definite information has been gained. The problem is beset with grave difficulties inherent in most attempts to determine coefficients of mutability. The possibility of overlooking even prominent variations until they have once been noted, together with the readiness with which they are found after one's attention has been focused on them, will hardly be questioned by anyone who has given attention to the discovery of new variations in almost any organism. One may attempt with some assurance an estimation of the frequency of recurrence of a particular mutation, for instance, whether it appears in vegetative parts of individuals or in sexually produced progenies, but it is a hazardous undertaking to estimate the frequency of variations in general. Until some one can devise a scheme for estimating the frequency of bud variations as Muller has done for determining mutation frequencies in *Drosophila,* little progress can be looked for other than through investigations of the somatic mutation or segregation of specific genes.

The problem of the relative frequency of occurrence of somatic and gametic variations meets the further difficulty that it is often impossible to determine the ontogenetic stage at which particular variations have arisen—a fact that has been noted for plants by various writers (deVries, 1910; Emerson, 1913; East, 1917). Both Bridges (1919) and Muller (1920) have discussed this problem from the standpoint of studies of particular mutations in *Drosophila.* The prevalent opinion that variations arise in the gametes or at about the time of their formation may have come in part from a belief that aberrant chromosome behavior is most likely to occur at the time of the reduction division. It seems likely, however, that the situation has been confused by failure to realize

that recessive mutations—the most frequent kind—can not be expressed in the individual in which they occur except when the dominant allelomorph is simplex, while such mutations may appear in a later generation of sexually produced progeny (East, 1917).

SOMATIC MUTATION OF GENES

Several cases of vegetative variation in plants have been studied with sufficient thoroughness to leave little doubt that they are mutations in the strict sense, involving the modification of particular genes. Most of them are concerned with variegated color patterns of flowers, leaves, or fruits, and they are more or less regularly recurrent, a fact that makes them especially well suited to quantitative studies, for it is obvious that a quantitative study can be made only of variations that occur with considerable frequency. For the most part also these somatic mutations are dominant to the type from which they spring, appearing frequently in material homozygous for their recessive allelomorphs, facts that exclude the possibility of their being due to any sort of somatic segregation of unlike genes. Blakeslee's (1920) case of a somatic variation in *Portulaca* is one of the few examples not involving variegation. Other cases have been reported by Baur (1918).

One of the earliest cases of somatic mutation was reported by deVries in variegated flowers of *Antirrhinum*. Though the work was done prior to the rediscovery of Mendelism and not discussed from the standpoint of recent genetic interpretation, there is little doubt, as I have noted elsewhere (Emerson, 1913), that the results can best be interpreted as due to a somatic gene mutation.

Correns's (1910) results with respect to the occurrence and behavior in inheritance of green-leaved variations on variegated-leaved *Mirabilis* and of self-colored flowers on variegated flowered strains of the same species were among the first to be subjected to critical genetic analysis. The behavior in inheritance of green branches of variegated *Mirabilis* shows this vegetative variation to be a simple dominant mutation affecting ordinarily only one of the duplex recessive allelomorphs. A mutated branch

is, therefore, as truly a heterozygote as if it had arisen through hybridization of green and variegated strains.

Self-colored branches on variegated-flowered plants of *Mirabilis* usually do not transmit the self-color character to their seed progenies in greater percentages than do variegated-flowered branches of the same plants. They are thought by Correns to be fundamentally of the same nature as the green branches of variegated-leaved plants, their failure to transmit the self-color character being due presumably to the accident that the mutation occurs in epidermal cells from which no gametes arise. The frequent occurrence of self-colored plants in seed progenies both of self-colored and of variegated flowers is considered evidence of their origin as vegetative rather than as gametic mutations, their failure of expression in the soma being thought due to their origin in sub-epidermal cells in which these flower colors do not develop.

Studies of variations in variegated pericarp of maize by myself (Emerson, 1914, 1917) and by Anderson, Eyster, and Demerec,[2] involve practically the same results as those so far reported in investigations of other species and afford in addition quantitative data on certain aspects of the somatic-mutation problem not included in other investigations. The genes for variegated pericarp have been shown to belong to a comparatively large series of multiple allelomorphs including those for colorlessness (white seeds), self color of different intensities, and certain definite color patterns of both the pericarp of the seeds and the glumes and paleæ of the cobs. Variegation is known to be a simple recessive to self color and a dominant to white.

Self-colored seeds whether occurring singly or in groups in variegated ears produce progenies consisting of approximately 50 per cent. self-colored ears, the other 50 per cent. being either all variegated or all white depending on whether the parent was homozygous variegated, *V V*, or heterozygous variegated, *V W*, from a previous cross with white. Seeds that are less than wholly self colored throw a correspondingly smaller per cent. of

[2] Unpublished data by W. H. Eyster and E. G. Anderson, and by E. G. Anderson and M. Demerec.

self-colored ears. Self-colored seeds thus produced have, so far as tested, proved to be heterozygous for self color, behaving in later generations exactly as if produced by crosses of self-colored with variegated or with white races.

Certain cultures of self-colored maize produce a few variegated seeds. Such seeds have been observed only on ears that are heterozygous from previous crosses with variegated strains, *S V*, or with white strains, *S W*, never from ears that are homozygous for self color, *S S*. From such variegated seeds, new variegated races have been produced.

These facts are regarded as indicating (1) that the occurrence of self-colored or partly self-colored seeds on variegated ears is due to somatic mutations of the recessive variegation gene to the dominant self-color allelomorph; (2) that only one of the two variegation genes of homozygous variegated maize mutates at a given time; (3) that it is always the variegation gene, never the white one, of heterozygous material that mutates; (4) that the occurrence of variegated seeds on otherwise self-colored ears is due to reverse mutations from the dominant self-color gene to the recessive variegation allelomorph; and (5) that only one of the duplex genes of self-color strains so mutates at any one time, for otherwise there would remain no dominant self-color gene to prevent the expression of the mutation as variegated seeds in homozygous self-colored material.

Another type of somatic variation, quite distinct from the self-color mutations discussed above and often termed dark-crown variation, also occurs frequently in variegated maize pericarp (Emerson, 1917). It is quite as striking in appearance as the self-color mutation, but is not inherited, the progenies of the aberrant seeds being in no way different from those of the normal seeds of the same ears. Microscopic examination of dark-crown and of self-color seeds indicates that in the former the epidermis alone is colored while in the latter the epidermis alone remains colorless. The conclusion seems warranted, therefore, that the two types of variation are fundamentally the same, both being true gene mutations, and

that the non-inheritance of the dark-crown type is due to the accident that it occurs in epidermal tissue outside the germ tract.

Recent investigations of variegated maize by Eyster and Anderson have established the fact that somatic mutations affecting small areas occur much more frequently than those affecting large areas. Since a mutation arising in a single cell late in development obviously could not affect so large an area as one originating earlier, it follows that mutations in variegated maize occur with increasing frequency in the later stages of ontogeny. It is true, as pointed out by Muller (1920), that given a constant rate of mutation throughout all stages of ontogeny and granting that one cell is as likely as another to mutate, mutations should appear more frequently in the later stages of development because of the fact that there are then many more cells in which mutations may arise. But Eyster and Anderson have found that the increase in the frequency of occurrence of mutations during the progress of development is accelerated far beyond expectation based on the increase in number of cells.

This behavior is strongly suggestive of a progressive acceleration in the mutability of the variegation gene as development proceeds. It is much too early to say whether this progressive change, if such it be, is inherent in the organization of the gene itself, as suggested by Anderson and Demerec, or whether it is a response to progressive changes in physiological and environmental relations. Perhaps the assumption of an equal chance of mutation as between any two cells is without sufficient warrant. Possibly there is a time element to be taken into account, as noted by Muller (1920). As cell division becomes progressively retarded in the late growth stages, may not each cell be exposed for an increasingly longer period of time to the chance of mutation? Perhaps it may be possible to test this assumption in favorable material by a comparison of the frequency of mutation in the very early slow-growth, the later rapid-growth, and the final slow-growth periods of the life cycle; but the relatively few cells present in the very early growth period seems likely to place serious limitations on the

practicability of such a test. An observation of possible importance in connection with the question of a time element in mutation and with the problem of environmental and physiological influences is that made by Eyster and Anderson concerning the greater frequency of the non-heritable (epidermal) mutations than of the heritable (sub-epidermal) ones in variegated pericarp of maize.

I have recently obtained results bearing on another phase of the somatic-mutation problem as related to variegated maize pericarp, namely, the relative frequency of mutation of homozygous, *V V*, and of heterozygous, *V W*, material. It has been shown above that the *W* gene for colorless (white) pericarp does not mutate, so far as known, when paired either with itself, *W W*, with the variegation gene, *V W*, or with the self-color gene, *S W*. It will be recalled further that only one of the two homologous genes in homozygous variegated, *V V*, material mutates at any one time. If it could be assumed that the mutability of either allelomorph is uninfluenced by the presence of the other, it should follow that somatic mutations will occur with approximately twice the frequency in homozygous, *V V*, as in heterozygous, *V W*, material. But this expectation has not been realized. On the contrary, both heritable (self-color) and non-heritable (dark-crown) mutations have appeared throughout all my cultures with somewhat greater frequency in heterozygous than in homozygous variegated ears. The difference has been especially pronounced in very light variegated strains, where mutations have appeared about two and one half times as often in heterozygous as in homozygous material. Even if mutations appeared with equal frequency in heterozygous and in homozygous ears, the simplex gene of the former must have a mutability of about twice that of either of the duplex genes of the latter. In the very light variegated strains, therefore, a simplex gene must have a mutability of about five times that of a duplex gene.

What appears to be a similar result in *Mirabilis* has been reported by Correns (1903, 1904). Crosses of a supposedly pure white race with several self-colored pink yellow, and pale yellow races resulted in every case in

plants with strongly red-striped flowers and with numerous self red flowers or even whole branches of such flowers. Intercrosses of the pink and yellow races gave only self-colored progeny, from which fact it was concluded that the white-flowered race carried a latent factor for striping. It was later discovered that about three per cent. of the flowers of the white race showed minute flecks of red. It was evidently an extremely light, variegated race, rarely if ever throwing somatic self-color mutations when the variegation gene was duplex (homozygous material) but producing such mutations with considerable frequency when that gene was simplex (heterozygous material). Correns concluded that red variegation of *Mirabilis* flowers is a character that, with self-fertilization or inbreeding, remains almost completely latent, but which, through the entrance of foreign germ plasms, is brought to full expression.

If the mutability of a gene can be increased through the influence of some modifying factor or factors brought into combination with it by crossing, as suggested by Correns, it should be possible to discover crosses that would not produce the effects so far observed in *Zea* and *Mirabilis*. While the problem deserves much more study from this viewpoint, it seems unlikely that results with maize can be explained on any such basis, unless the postulated modifying factor is the allelomorph of the variegation gene or some factor very closely linked with it. It must be noted in this connection that the comparison in maize was made between homozygous and heterozygous variegated ears of the same F_2 progenies grown from self-pollinated F_1 heterozygotes—a circumstance that would afford abundant opportunity for recombinations of independently inherited modifying factors. That the differences in mutability noted in maize may be due to differences in the interaction of like as contrasted with that of unlike allelomorphs, as suggested by Anderson and Demerec, is a somewhat novel conception worth careful consideration if means can be devised for subjecting it to a crucial test.

Before the topic of somatic mutation is dismissed, it should be noted that the phenomenon is not limited to

plants. Among animals, *Drosophila* (Morgan and Bridges, 1919) has furnished several examples of undoubted somatic mutation resulting in mosaic individuals other than gynandromorphs.

SOMATIC SEGREGATION

Bud variations have probably been ascribed to somatic segregation more frequently than to any one other cause. Perhaps the opinion commonly held that bud variations occur more frequently in hybrids than in other material and the long known fact that seed-grown offspring of hybrids exhibit segregation, is chiefly responsible for this usage. It is, of course, possible that most vegetative variations are of this nature, but the fact that the individuals in which they arise are frequently found to be heterozygous for the genes concerned is no conclusive evidence that segregation is involved. Mutations also, as noted by several writers, are most likely to appear in heterozygous material because most of them are recessive and the unmutated dominant allelomorphs prevent their expression in the individuals in which they originate if the latter are homozygous.

Chromosome Elimination.—The best examples of somatic segregation that have been subjected to critical genetic analysis are afforded by the work with *Drosophila.* It has been shown by Morgan and Bridges (1919) that, of the relatively numerous gynandromorphs which have appeared in the course of investigations with *Drosophila,* nearly all have resulted from the elimination of the sex chromosome at some early cleavage division. If a fertilized egg starts as a female, XX, and one X chromosome is eliminated at an early segmentation that part of the individual developing from the cell that receives but one X chromosome should be male, XO, while the remaining part should be female, XX.

The evidence in support of this view was obtained from crosses the parents of which had different sex-linked and different autosomal characters, that is, characters whose genes are carried by the sex chromosomes and by the autosomes, respectively. The male, as well as the female,

side of gynandromorphs appearing in such crosses exhibited all the dominant autosomal characters whether they came from the maternal or the paternal parent. When the mother had a recessive, mutant gene in one of her autosomes and the father had its dominant, normal allelomorph, the fact that the male side of gynandromorphs did not have the maternal, recessive autosomal character effectively disposed of Boveri's hypothesis of partial fertilization. On the other hand, when a recessive autosomal gene entered from the father's side and its dominant allelomorph from the mother's side, the fact that the male side of the gynandromorphs did not show the paternal, recessive character likewise eliminated Morgan's earlier hypothesis of polyspermic fertilization. It has been shown, further, from crosses, the parents of which differed in sex-linked characters, that maternal and paternal X chromosomes are eliminated with about equal frequency.

In certain experiments with *Drosophila,* in which a determination of the frequency of sex-chromosome elimination was undertaken, it was found that one gynandromorph appeared in about every 2,200 individuals. Since only those individuals that start as females give the kind of gynandromorphs observed in these tests, it was concluded that one case of chromosome elimination occurs in about 1,100 individuals.

Of the evidence from plant material there is the recent account by Frost (1921) of the occurrence of a bud sport in *Matthiola* in which presumably linked genes have segregated out simultaneously in one or more branches. While this case will require further investigation before the manner of its origin can be positively established, it seems probable that it belongs to the category of somatic segregation by chromosome elimination or non-disjunction.

Studies of mosaic endosperm of maize afford perhaps the most definite evidence available in plants that certain somatic variations are due to aberrant chromosome behavior such as non-disjunction or elimination (Emerson, 1921). The genetic evidence that I have been able to obtain in support of this interpretation is of much the same

nature as that noted above for *Drosophila* gynandromorphs. In crosses in which recessive aleurone and endosperm characters are contributed by the female parent and their dominant allelomorphs by the male parent, spots of the recessive (maternal) aleurone color are underlaid by the recessive (maternal) type of endosperm when the genes for these aleurone and endosperm characters are genetically linked, that is, when they are carried in the same chromosome. On the contrary, similar recessive (maternal) aleurone-color spots are always underlaid by the dominant (paternal) type of endosperm when the genes are not linked, that is, when they are carried in non-homologous chromosomes. The fact that linked genes separate out simultaneously while non-linked ones do not do so supports the view that mosaic seeds are the result of some chromosome aberration such as elimination or non-disjunction, and renders untenable the earlier hypotheses of incomplete fusion of endosperm nuclei suggested by Correns and by Webber and also that of gene mutation proposed by myself.

The work with aberrant maize endosperm has furnished an opportunity to study the frequency of chromosome aberrations in a specialized tissue. The available data show that when a single chromosome alone is concerned, about one mosaic seed occurs in every 420 seeds. If the other two homologous chromosomes of any one set are involved as frequently and if any one of the ten triploid chromosome sets is as likely to be involved as any other one, one case of aberrant chromosome behavior should occur in about every fourteen seeds. There is some evidence, though not convincing as yet, that in different strains of maize chromosome aberrations may occur with strikingly different frequencies. In one culture in which only a single chromosome could have been involved in the origin of mosaic seeds, as many as twenty-five such seeds have been observed on a single ear of approximately 500 seeds, or one for each 20 seeds. If this behavior proves to be a constant one in this strain and if the other 29 chromosomes behave in like manner, it should furnish excellent material for cytological investigation. Moreover, the possibility of the existence of

strains of maize differing so widely in the frequency of chromosome elimination or non-disjunction raises interesting questions concerning the causes of such aberrations. It would seem possible to determine by appropriate tests something as to the relative influence of maternal and of paternal contributions on the rate of chromosome elimination.

There are circumstances connected with these results from *Drosophila* and *Zea* that may raise some doubt of their general applicability to cases of bud variation. The *Drosophila* evidence is limited almost exclusively to the sex chromosomes, though there is no positive evidence that elimination may not occur among autosomes and result in non-viable individuals. The data from *Zea* relates to endosperm alone, a specialized, nutritive, sterile, triploid tissue. There is perhaps justification for a belief that the sex chromosomes of animals and the triploid chromosomes of the endosperm of angiosperms may be subject to irregularities in behavior not commonly found in other material. The only answer to such a contention is (1) that gynandromorphs and endosperm mosaics are the materials that have been critically studied and (2) that there is, or should be, no presumption in favor of vegetative segregation through chromosome elimination or through other means as against vegetative mutation or any other mechanism as a possible explanation of bud variations that have not been subjected to cytological investigation or to critical genetic analysis.

Cytoplasmic Segregation.—Numerous cases of apparent segregation of cytoplasmic elements have been reported in plants. Of these, examples from *Mirabilis, Pelargonium, Primula,* and *Zea* may be noted. All of them involve visible effects on chlorophyll development and all show non-Mendelian inheritance.

Correns (1909*a, b*) working with a white-spotted-leaved type of *Mirabilis* observed a very irregular distribution of the white and green areas, each varying from small spots to whole branches. These white and green characters were found to be inherited through the mother only. The situation with respect to *Pelargonium,* re-

ported by Baur (1909), differs from that in *Mirabilis* in that the spotting is transmitted through the pollen as well as through the egg cells. Spotting appeared in F_1 in crosses of white with green without respect to which way the cross was made. As in *Mirabilis,* wholly white and wholly green, as well as mosaic, branches were observed.

Examples of maternally inherited chlorophyll variegation have been investigated by Gregory (1915) in *Primula,* and by Anderson[3] in *Zea.* The genetic behavior of these materials is quite the same as that of Correns's *Mirabilis* variegation. The apparent difference in the cytological basis of their behavior, however, must not be overlooked.

Evidently these plants of *Mirabilis, Pelargonium, Primula,* and *Zea* are sectorial chimæras. Their main interest in connection with this discussion lies in the fact that, starting with a single fertilized egg cell, certain chlorophyll deficiencies are apparently separated out into certain vegetative cells and handed on through definite cell lines, while normal chlorophyll develops in other cell lines, with the result that areas of varying extent have one or the other of these characters. In what the mechanism of this segregation consists—if segregation it be—is not in all cases certainly known. It may even be that some cases of variegated chlorophyll are to be regarded as recurrent variations arising *de novo* after the manner of somatic mutations but effecting changes in the cytoplasm, or some of its inclusions, rather than in the chromosomes. Baur is inclined to the view that in mosaic plants of *Pelargonium* deformed chloroplasts are responsible for the chlorophyll deficiencies and that these are segregated out by chance in cell division. This view is supported by Gregory, who noted in the young leaves of variegated plants of *Primula* the existence of normal and chlorotic plastids in the same cells. Correns does not commit himself to any particular element or inclusion of cytoplasm as the seat of the cause of chlorophyll deficiency. Randolph (1922), from cytological examination of Anderson's striped leaved maize, found that, in the

[3] Unpublished data.

transition regions between the green and the pale-green areas, the cells contain not only green and colorless plastids, but all intermediate conditions as well. Since the green and the white plastids are not two sharply differentiated kinds, but are the end members of a continuous series arising from minute primordia which, so far as can be seen, are of one kind, he regards any simple form of segregation hypothesis as inadequate. It seems possible, however, that these primordia may be functionally, even though not morphologically, of two more or less distinct classes.

Graft-hybrids and Other Chimæras.—The well-known graft-hybrids of *Solanum* reported by Winkler are of interest from the standpoint of this discussion because of the bud variations commonly exhibited by them. Sectorial chimæras, produced by adventitious buds arising from the point of union of stock and scion of grafts of tomato and nightshade, and having one side of the one species and the other side of the other, have not infrequently later produced branches that were periclinal chimæras having tissues of one species enclosed within an envelope of the other. That these branches are really periclinal chimæras has been established by chromosome counts and by the fact that seedlings produced by them are always of the species of the subepidermal tissue from which gametes arise. These periclinal chimæras in turn have been observed to produce branches wholly of one or other of the parent species. The marked difference in appearance between the sectorial and periclinal chimæras and between the latter and either parent species places this behavior clearly in the class of bud variation and, since the production of branches of the parent species from periclinal chimæras is the result of a separation of genotypes that were closely united previously, the phenomenon is perhaps rightly classed as a form of vegetative segregation. It is obvious, however, that the separation of tissues that are merely closely associated in the graft hybrid is a fundamentally different type of segregation from that by which the chromosomes or even the

plastids or other cytoplasmic elements of a single cell are dissociated.

The behavior of "natural" periclinal chimæras of *Pelargonium,* noted by Baur (1909), and of *Pelargonium* and several other forms, described by Bateson (1919), all of which involve green and white regions of the plants and some of which produce reverse periclinal chimæras, is fundamentally the same as that of graft-hybrids. The manner of origin of these natural chimæras is unknown, but it is quite possible that they arose as somatic mutations.

The case of *Bouvardia* also, as reported by Bateson (1916), is presumably of quite the same order as the examples noted above, though its behavior is strikingly different in detail. Varieties of *Bouvardia* that are maintained true to type by propagation from stem cuttings produce plants with very different flower form, size, and color when propagated by root cuttings. While this behavior is not to be taken as positive proof that these varieties are natural periclinal chimæras, it is quite in keeping with such an assumption. Since in normally produced buds of the stem both the epidermis and the deeper lying tissues are maintained through direct cell lineage, while the roots produced by stem cuttings arise from the plerome and break through the periblem and dermatogen, forming these parts anew, sprouts that develop from the roots must have the genotype of the stele rather than that of the cortex or epidermis.

From the results of critical investigations cited in this account, it is evident that vegetative variations are due to diverse causes. Some are certainly due to somatic mutation of genes; others are as certainly due to chromosome aberrations; and still others have been somewhat definitely shown to involve a vegetative segregation of plastids or other cytoplasmic elements. There are many problems relating to these several types of behavior that are in great need of further critical study both genetic and cytological. The results of future research will depend in large measure on the choice of favorable material. Quantitative data are of the greatest importance

and from this standpoint no material gives more promise of fruitful results than that involving variegation.

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SEROLOGICAL REACTIONS AS A PROBABLE CAUSE OF VARIATIONS

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WITH an insight that has never been surpassed even to this day Claude Bernard,[1] more than forty years ago, remarked that "Organic synthesis, generation, regeneration, maintenance, and healing of wounds, are different aspects of an identical phenomenon," the phenomenon alluded to being the constructive activity manifested in ordinary nutritive processes. In a recent thoughtful paper, R. S. Lillie[2] reiterates and expands this point of view. That synthetic metabolism constitutes the very essence of embryonic development and therefore of the expression of heredity, scarcely admits of a doubt. Today it is a truism to say that the visible "characters" we deal with in heredity are but the effects—by-products as it were—of far-reaching metabolic reactions. And since the metabolism of the actual living protoplasm centers, if not exclusively, at least principally, in the proteins, the problems of metabolism, growth, reproduction and heredity become largely the problem of why and how a given kind of living protoplasm builds up proteins of its own specific type.

The molecules of the ordinary native proteins are, as is well known, huge polymeric structures of extremely complex constitution. By appropriate chemical treatment they may be broken down into successively smaller and smaller units, each of which, however, still responds to the ordinary qualitative tests for proteins. There finally comes a point below which further reduction of the molecule results in the loss of the distinctive protein reaction, and the outcome is a series of ultimate characteristic units, the amino-acids. Thus native proteins seem to be built up of two different categories of units: first, combinations of various amino-acids which constitute the simplest protein blocks; and second, the combination of these into the much larger molecules characteristic of the native proteins.

[1] "Leçons sur les phénomènes de la vie," Vol. II, p. 517.

[2] *Biol. Bull.*, XXXIV, 2, 1918.

In the process of digestion different proteins are broken down into their amino-acid units and these are then rebuilt into the tissue-proteins of the living organism, each tissue selecting such amino-acids as are required to reconstruct its own peculiar complex. That is, the architecture of the new proteins into which the individual building units are regrouped is determined by the specific constitution of the tissue-proteins themselves. In different proteins the different amino-acids may exist in very different ratios, and certain of them necessary for the metabolic repair of protoplasm may be lacking in some, such as gelatin, but the amino-acids in any particular protein are constant in nature and proportion and each probably has a definite position in the molecule. While kinds and proportions of amino-acid units determine in large measure the characteristics of individual proteins, it may well be that configurational differences in molecules of the same chemical composition are responsible for the specificities of corresponding proteins in related species of animals. One estimate, for instance, assigns to the serum-albumin molecule alone the capability of having as many as ten thousand million stereoisomers. One may perhaps picture mentally, in a much simplified form, the simplest protein molecule as a main chain or ring, of which the representative links are amino-acid "nuclei." Moreover, to each such link

(*e.g.*, in simplest form, —NH.CH.CO—)
|
H

a side-chain, differing in constitution in different cases, is attached or is attachable by replacement of a hydrogen atom.

It is, then, with such complex molecular configurations that we have to do as a chemical basis for the phenomena of life; and in them, as I have stated elsewhere,[3] we have "ample basis for that peculiar handing on of metabolic energies already established which we term *heredity*."

Although habitually when speaking of heredity we think of the multifarious "characters" displayed by the adult organism as the things inherited, and strive to picture in our minds how they are represented in the germ, it is

[3] AMER. NAT., XLV, May, 1911.

clear, in the light of modern genetics, embryology and cytology, that what actually happens is a reduplication generation after generation of germinal protoplasm. That is, the proteins already present in the germ-cell not only determine what will be built up in growth, but also the composition of that overgrowth which, as a detached individual, constitutes the physical basis of inheritance. If the initial protoplasmic substance is chemically specific then inevitably the anatomical and physiological complexities which arise out of it must likewise be specific.

Before we can grapple with the problem of the possible induction of changes in this fundamental mechanism through influences emanating from the body, we must consider some matters concerned with embryonic development and the fundamental chemical nature of the somatic cells.

As to how the constitution of the egg becomes transformed into that of the adult, the most consistent and reasonable hypothesis to date, in my opinion, is that proposed by Child, based on axial or metabolic gradients. A full exposition of his hypothesis must be sought in his books, "Individuality in Organisms" and "The Origin and Development of the Nervous System from a Physiological Viewpoint." I can sketch only such aspects of it as pertain to my present subject. Starting with the universally accepted biological axiom that excitability followed by some degree of transmissibility is a fundamental property of all living matter, Child believes, as I understand him, that the establishment of polarity in the fundamental organismic proteins of the germ-cell is the beginning of development. The eggs of many species already show polarity (animal and vegetal pole) at the time of ovulation; in other forms polarity is not established until later. In the former case the polarity may have been determined in earlier cell-generations by extrinsic factors or it may be due to the original position of the ovum in the ovary with reference to the nutritive stream. Yolk apparently accumulates in the region of least oxidation and thus marks the vegetal pole. In the second type of egg, polarity, at first lacking, is soon established because differential environmental exposure (difference in oxygen supply, light, contact, general surface exposure,

or other external factor or factors) causes one region to have the greatest metabolic activity. As does any stimulated part with reference to a resting part (muscle, nerve, etc.), this point of heightened activity sets the pace, as it were, for the other parts. Thus an excitation gradient is established which Child terms an *axial gradient.* Since the excitation initiates transmission—changes and thereby determines what shall happen at successive levels along the path of transmission, the region of highest excitation dominating and controlling the rest, the axis in question may also be called a *metabolic gradient.* In this way a physiological unity is established and maintained by bringing the different regions within range of the gradient into definite physiological relations.

Since the chief source of energy in protoplasm is oxidation, and inasmuch as many different tests have shown that the rate of oxidation gradually diminishes along the gradient from the region of highest activity, Child infers that differences in oxygen supply play a very important part in the local metabolic differences which arise. In any event, a differential axis of activity arises in the egg as the result of differences in environmental conditions (either before or after ovulation) and determines the axiate pattern of the developing organism. In such a complex system of chemical and physical activities, where unquestionably many associated simultaneous reactions and interactions are going on, different rates or conditions of reaction in different regions must result in unlike end-products. Thus, at different levels of a gradient which was quantitative in origin, *qualitative* differences arise. For once a gradient is established, any one of several purely quantitative changes in the system, such as increased oxidation which acts differentially on substances at a given point, changes in temperature, in water content, or in colloidal state, or changes in the concentration of the reacting substances, may alter certain component factors of a given region more than it does the corresponding factors in other regions, with the result that out of the same initial constituents the respective organ- or tissue-stuffs that characterize the organism are gradually built up. For example, as Child points out,

in a region of rapid oxidation certain substances might be entirely oxidized as rapidly as they are formed, while in a region of slower oxidation they might accumulate as part of the structure.

Bilateral organisms follow a law of antero-posterior development. Differential exposure having determined which region shall lead and having thus set the rate of activity of the successive regions, continuing differential relations of the environment maintain the various levels of the gradient, under the domination of the head-end, at their respective rates of activity. While this is the normal course of development, it may be greatly altered by experimental methods; the original gradient, particularly in lower organisms, may be obliterated and a new one engendered. The latter under certain conditions may even be made to arise at right angles to the original axis. Change of gradient may be readily observed, for example, in the regulatory development of isolated pieces of many planarians. Moreover, the remarkable capacity for self-differentiation possessed by isolated parts taken at different levels of the body in such forms, shows that positional relations of the constituents of the regenerating mass, rather than cellular specificity, determine what structures shall arise in a given location.

Since many species, including representatives from all the chief phyla of animals, show differential susceptibility at some stage of their development, when subjected to the action of various external agents such as alcohol, anæsthetics or potassium cyanide, these agents may be used to bring about shifts of gradient, under-development or over-development of certain parts, or a remodelling of the organism or of various regions of it. Inasmuch as any one of several agents may bring about the same result, it is manifest, again, that quantitative external conditions are the factors which initiate and thereby determine the fundamental orientations and specializations of the parts of an organism.

As development progresses in the more complex organisms, again through differential stimulation, secondary or "symmetry" gradients may also become established. For example, each limb region of a vertebrate becomes a subordinate system with its own internal correlations.

In organisms with radial symmetry, special centers of growth occur, and only at a certain distance from a given center can another arise.

After differences in protoplasmic constitution have arisen at different levels of the gradient, a system of chemical or transportative correlation probably begins to operate, and out of it all finally comes the various supportive and mechanical tissues, vascular tissues, tissues of excretion and secretion, nervous tissues, etc., which constitute the underlying mechanisms of correlation and integration in the finished organism.

The hypothesis says little of chromosomes, or of genes, for its objective differs somewhat from that of the geneticist, but it in no wise denies the existence of such entities. Child argues, however, that since each cell of the body is a descendant of the original zygote and therefore presumably possesses the full complement of chromosomes of that zygote, something other than the nuclear pattern *per se* must be responsible for the fact that cells become different in different parts of the body. And this something, he would say, is the metabolic gradient initiated by differential excitation, since the very establishment of such a gradient means the concomitant establishment of local differences along its path. As he says, "if all the cells are originally alike they cannot of themselves become different." The specific character of the differentiation, the kind of organ or organism produced, he reiterates, is determined "by the specific inherited constitution of the protoplasm."

So much for Child's hypothesis of axial or metabolic gradients, in its bearings on embryogeny and differentiation. I have reviewed it at some length because it shows more clearly than any other theory of development with which I am acquainted that there is no necessity for believing that as cells become specialized they lose part of their original constituents. Due to local conditions, structural modifications and special activities have appeared, but these are changes rung on what is fundamentally the same type of protoplasm in every cell. In higher organisms, in some cells possibly irreversible changes have occurred—the cell may be incapable of dedifferentiation —but nothing constitutional, call it gene or what you will,

has necessarily been lost from the cell. The inheritance complex of the germ is like goods in the piece; it is only as development progresses that the garment becomes specified; but above all, be it remembered that the finished garment is of the same fundamental constitution as the goods in the piece.

It is a commonplace of experimental embryology and experimental morphology, in fact, that the same initial materials may yield very different end-products in different environments. The phenomena of heteromorphosis, metaplasia, regeneration and regulation all attest this. Blastomeres originally directed toward becoming one part of an organism may be switched about to become another part; tissues originally subserving one function may be turned to other uses; ectodermal cells which by no possible chance could have been predestined to form crystalline lens will, nevertheless, form a lens similar to that of the normal eye if stimulated by a transplanted optic cup. Or, if the lens is removed from the eye of a salamander, a new lens may develop from the edge of the old iris, a part from which the lens never normally develops in the embryo. In short, it is a well-established fact in many organisms that cells occupied with the specializations of one part of the individual still retain the potentialities which would fit them to the functions of some different part, and may, in fact, under experimental conditions be made to redifferentiate into the structures of another part. Such facts, together with the exactitude of chromosome distribution in mitosis, indicate clearly that many, possibly all, cells of an organism retain the hereditary tendencies that existed in the original zygote. Because of limitations due to its location in the organism, however, a given cell realizes only a small proportion of its inherent possibilities. And after all, this is no more remarkable than the fact that the genes of recessive characters may slumber indefinitely in germ and soma, generation after generation, until conditions suitable for their expression as characters occur.

But now, regarding heredity as in its simplest expression merely the passing on of metabolic activities already established, and conceding that the distinctive structural

effects and functions which characterize the respective tissues are probably the outcome of unequal activities among the same kinds of fundamental protoplasmic constituents in differing local environments, the question of prime importance to the student of evolution is how the properties of these constituents have come to be changed from what they were initially, how they may be altered in the future—in short, the question of the nature and origin of variations. For whatever we may believe about the degree of preformation which exists to-day in the mechanism of heredity, it is absurd to assume that in the simpler primitive protoplasm from which modern forms have evolved there could have been genes of the characteristics of all the organisms now in existence. Whatever individual development may be, we must assume that racial evolution was epigenetic. While doubtless in a sense man lived potentially in some primitive protozoan-like creature, actual material antecedents of his existing attributes were no more present in this ancestral creature than specific determiners for the oceans, continents and topographical features of the world to-day were present in the original nebula which preceded our solar system. The great central problem of evolution is just this very one of how the determinative accumulations which exist in germ-cells to-day have been incorporated step by step into this erstwhile primitive protoplasm. Certain possibilities have become realities and concomitantly as a basis of this reality the old mechanism has in part been altered, or a new mechanism has come into being which persists as a part of the established constitution of the germ-cell.

Before entering upon a discussion of whether or not any of the remarkable serological activities which have come to light in recent years may be possible or probable sources of germinal modifications, we must recall briefly the general nature of immunologic reactions. As you know, foreign proteins of either plant or animal origin when injected directly or indirectly into the circulation of an animal will engender antagonistic or neutralizing substances to which the general name of *antibodies* is applied. Thus the toxins of bacteria incite the production of *antitoxins;* the bacteria themselves lead to the pro-

duction of bacterial immobilizers or solvents termed *bacteriolysins,* or sometimes to agglutinating substances termed *agglutinins* which clump bacteria of the species used in their production, if the two are brought together in the blood-serum of the animal into which the bacteria were originally introduced. Likewise a tissue of one kind of animal injected into the circulation of another induces the formation of antibodies of various kinds such as *precipitins* which form a precipitate when the blood-serum of the treated animal and an extract of the special tissue used are brought together in vitro; or other antibodies termed *cytotoxins* or *cytolysins* which possess a specific toxic or solvent action for the kind of protein used in their production. The alien substance employed to produce antibodies is commonly called the *antigen.*

In this connection, the phenomenon of anaphylaxis should perhaps also be mentioned. Anaphylaxis is a name given by Richet to designate a highly supersensitive state which, after a period of incubation, an animal develops toward certain protein substances that were practically harmless on first injection. Sometimes, particularly in guinea pigs, death results. The sensitizing dose for the production of anaphylaxis may be very small; one millionth cubic centimeter of horse-serum, for example, has been known to render guinea pigs sensitive. The reaction is specific; for instance, an animal sensitized to sheep-serum, though reacting violently to this antigen, displays little or no hypersusceptibility to other sera.

In the main all of the immunological reactions show a considerable degree of specificity; the antibody will react fully only with the particular kind of protein used as antigen. The specificity is not absolute, however; a milder reaction may be obtained with homologous proteins of related species, the extent of the reaction being determined by the nearness of relationship of the species to that from which the original antigen was obtained. Similarly with bacteria, the reaction is in the main specific, although so-called group-reactions may appear. The serum of an animal immunized against typhoid, for example, may not only agglutinate *Bacillus typhosus* but may also show this reaction in a less degree with such related forms as the colon bacillus. Thus, ir-

respective of whether the antigen consists of bacteria or of other protein materials, there is a gradational specificity of reactions which apparently corresponds to taxonomic relationships.

An even more delicate biochemical measure of kinship than the known immunological reactions has apparently been established through the extensive researches of Leo Loeb[4] and his associates on transplanted tissues. In numerous of his papers Loeb has called attention to the remarkable power of transplanted tissues to indicate different degrees of even close individual relationship, such as the individual to itself, to a brother or sister, to a parent, to a more distantly related individual of the same species, or to an individual of a different species. Particularly the lymphocytes of the host serve as a delicate indicator of such relationships.

Loeb assumes that a specific chemical group which he designates as *individuality-differential* is common to all the tissues of an individual and that in virtue of this characteristic each creature differs from the others of the same species. The individuality-differential of a transplant (except in autotransplantation), since it is not adapted to its new environment, assumes injurious properties, probably by engendering toxins. The relative strengths of these are determined by the degree of relationship that exists between the source of the transplant and the host. In the circulating fluids of a given individual, he believes that there are "autosubstances" which exercise important regulative functions, such, for example, as keeping the vascular supply of the various tissues at an optimum, or holding in check lymphocytes and invasive fibroblasts which when inadequately restrained, as in old age, become destructive agents. In one place he speaks of their stimulating effects and he regards them as responsible directly or indirectly for the marked vascular reaction called forth by autotransplants.

In sexual reproduction, obviously two different "individuality-differentials" must combine to form the new individuality-differentials of the offspring. These, Loeb finds, are of varying degrees of intermediacy. This intermediacy is continued into the next generation. What

[4] AMER. NAT., LIV, 1920.

is of much interest from the standpoint of our quest of a possible connection between reaction-products of the body and alterations of the germ is the fact that he feels constrained to link up his serumal phenomena with the chromosomes. Thus, he says, "The chemical individuality-character of the chromosomes should lead to analogous chemical differences consisting perhaps in the formation of chemical side-chains attached to proteins; they should be present primarily in cell-proteins and secondarily in the proteins of the body-fluids. . . . These side-chains must be identical in all the proteins of the same individual and differ in the case of different individuals."

Another great group of influences which extend to the furthermost reaches of the body and profoundly affect the entire organism in development and in maturity—those emanating from the various endocrine structures—I have barely time to mention. They must be kept in mind, however, when we attempt to picture the ebb and flow of chemical influence which is indispensable to the maintenance of general physiological equilibrium, including that of the gonads no less than of the other body structures.

You may feel that in reviewing the nature of the protein molecule, the behavior of the proteins of the cells in morphogenesis, the gradational specificities of the immunological reactions, the relationships which exist between host and transplant, and in reminding you of the intricate functions of the endocrines, I have wandered far afield into irrelevant byways, but I hasten to assert that these phenomena are not as unrelated as might appear at first sight; they are but different aspects of the great salient fact of organismic unity, whether it be a matter of chemical constitution, taxonomic relationship or physiologic response.

And now I wish to raise the question of whether or not in the light of the foregoing facts it is irrational to believe that in all probability a thread of chemical identity persists between the chemical constituents of the germ and the chemical substratum of the tissue-cells. The nuclei of the various tissue-cells differ little in appearance from the nuclei of the germ-cells, and inasmuch as the new germinal and somatic cells descend alike

directly from a common source, presumably bearing in their chromosomes samples of all the chromosomal components of the original zygote, is it unreasonable to suppose that if changes come to pass which can affect certain constituents of tissue-cells, this influence, if borne in the circulating fluids of the body, could also affect the homologous constituents of the germ-cells? Personally, I think that such a hypothesis is not unreasonable. But is there even the least bit of evidence on this point? I believe that there is. I feel that in the transmission of eye-defects secured by Dr. E. A. Smith and myself in fetal rabbits by means of serum immunized against rabbit crystalline lens, we have a bona fide case of such parallel influences. Since I have already presented the facts before this Society and inasmuch as the details are available in printed form,[5] I need not repeat them now. It is sufficient to recall to you that we secured a fowl-serum immunized against rabbit crystalline lens which when injected into pregnant rabbits penetrated the placenta and occasionally attacked the lens of the fetal young, the outcome being marked eye-anomalies in such young. Since, once produced, the defects were transmitted to successive generations through both male and female lines, we interpreted our results to mean that the immune serum was not only specifically cytolytic for the newly forming lens-tissue of the fetus, but that it also attacked the representatives of such tissue—its genes, if you please —in at least some of the germ-cells of the fetus. If true, this must mean that there is some degree of constitutional identity, probably protein homology, between the mature substance of a tissue and its correlative in the germ. And in view of the fact that, basically, inheritance is mainly a question of the perpetuation of specific protein-complexes, and development, the result of differential reactions of these same fundamental constituents under differing conditions of environment, is this an unreasonable inference?

But does anything comparable to this occur in the ordinary course of animal existence? Do cytolysins or kindred substances which can modify or destroy both

[5] *Jour. Exp. Zool.*, 31, 2, 1920. AMER. NAT., LV, 1921. *Proc. Nat. Acad. Sci.*, 6, 3, 1920.

tissue-elements and their germinal correlatives ever occur in animals without being introduced by man? Do animals ever form such antibodies or other equally active substances against their own tissues? It is obvious, since tissues persist intact under conditions of normal physiological equilibrium, that they are not being subjected to such influences, or if they are, that they resist them. As a matter of fact, Römer,[6] using the complement-fixation technique, found that the serum of adult human beings may possess antibodies for their own lens proteins. It seems reasonable to suppose that if the tissues of an animal became injured or displaced in some way, or metabolically unbalanced, immunity reactions might be established against them. We have some evidence that such is the case. During the late war, for example, it was found that toxic reactions resembling anaphylactic shock often followed extensive injuries of the soft tissues. The matter can be tested experimentally. Because of their distinctive nature and the ease with which they may be isolated, I chose spermatozoa for such an experiment.[7] I found that a rabbit will build antibodies against its own spermatozoa when these are injected into its blood-stream; also, that rabbits injected with rabbit spermatozoa not only develop antibodies in their blood, but also have their own spermatozoa greatly weakened, a condition shown in vitro by their lessened resistance to antisera. This clearly shows that an animal can on occasion build antibodies against its own tissues; and since antibodies can apparently directly or indirectly affect germ-cells, it seems reasonable to suppose that such influences, especially if continued over a long period of time, might be one source of germinal variations.

It is known from the experiments of Kuntz[8] and others that the blocking off of the ductus deferens of one testis may induce degeneration of the germinal epithelium, not only of that testis, but of the other as well. Inasmuch as the spermatozoa in the testis on the operated side must die and be resorbed, is it not probable that in this process spermatotoxins have been formed which have then attacked the living germ-cells of the other testis? Again, we are familiar with the fact that oculists fre-

[6] Zinsser: "Infection and Resistance."

[7] Paper in press, *Jour. Exp. Zool.*

[8] *Anat. Rec.*, 17, 4, 1919.

quently find it necessary to remove a severely injured eye to prevent the "sympathetic" degeneration of the other eye. I am told by competent oculists that the extension of the degenerative influence involves more than the atrophic effects which might result through direct nerve connections. Does it not seem probable that here, too, the disintegrative influence which comes to operate on the uninjured eye is cytotoxic or cytolytic in nature? And if it can operate on the tissues of the normal eye, why not on the corresponding protein constituents in the germ, the prototypes of those which were originally incited to form the ocular tissues?

It may be, it probably is true that there is sufficient difference between these factors of the germinal protoplasm and those of the finished organ to render the former less susceptible to such agents. It is not improbable that even if some of the numerous germ-cells were affected many others might not be. But any new organism which sprang from such an affected germ would have its own germ-cells similarly modified, since these would all be derived from the same zygote. Even so, the defects might not be manifested in offspring because of the probability of dominance by the corresponding factors from their partner in fertilization.

The only way to settle the matter, of course, is through experiment. I know of no existing experimental evidence on this point. In my own laboratory, however, an investigator has an experiment in progress which I hope will ultimately throw some light on the matter.

There are many bits of evidence to show that an organism may react against the tissues of other individuals of its own species. Thus Bradley and Sansum,[9] employing anaphylactic reactions, found that guinea pigs injected with various guinea-pig tissues such as heart, liver, muscle, testicle, and kidney developed immunity reactions. Moreover, certain changes in the blood of the mother during pregnancy, apparently induced by cells or cell-products set free from the newly-forming placenta, seem to be of the nature of antibody formation. Then again Turck[10] has shown that products of the lung-tissue of the cat, autolysed under sterile conditions in *vitro*, pro-

[9] *Jour. Biol. Chem.*, Vol. 18, 1914.

[10] *Med. Rec.*, 1919, 95, pp. 719–21.

duced characteristic pulmonary lesions when injected into other cats. Similarly autolysed lung-tissue of other mammals had no effect on cats.

But, the question arises, in order to get parallel influences, in soma and germ, would there not have to be absolute identity between the two sets of proteins concerned? Before answering this question let us glance for a minute at two types of specificity which are recognized in serological reactions: namely, "species-specificity" and "organ-specificity." What the serologist means by species-specificity is the fact, shown through precipitin reactions, that blood immunized against one tissue of an alien species will react, although in a less degree, with extracts of the other tissues of that species. And that there may be a specificity of certain organ complexes which is independent of species is shown by the fact that an immune serum produced by using the crystalline lens of one species of animal yields a precipitin which reacts more or less with the lens proteins of even unrelated species. Similar results have been obtained with proteins derived from the testis, and confirmatory evidence of such organ-specificity has also been established by means of anaphylactic reactions. Such facts as these, together with those cited in the discussion of the gradational reactions of various immune sera according to the systematic relationships of animals, it seems to me, answer our question affirmatively; there need not be absolute identity between the proteins of the somatic cells and their correlatives in the germ-cells for immune sera engendered against the one to react also against the other. I raise the issue because it might be urged that such tissues of an organism as become so abnormal as to excite the production of antibodies are no longer sufficiently similar to the normal tissue-elements, and therefore to their germinal representatives, to make the antibodies effective against either normal somatic or germinal constituents.

It seems to me that all available facts indicate that the constitution of an organism, whether germ or soma, is not to be regarded as a congeries of cooperating, equipotent units, but rather as the outcome of interacting systems which differ in their orders of organization; systems which in themselves possess more fundamental and

more supplementary or fluctuating components; chemical groups which represent the more constant features of organization coupled with subsidiary groups of more restricted significance. That is, there seem to be series of substances of like chemical constitution common to all the cells of an organism, possibly to even various groups of organisms, and superimposed upon these central or foundational constituents, probably as parts of the same molecules, are secondary systems, or possibly systems within systems, which modify the main configurations in various ways.

This conception certainly squares with the fact that degrees of specificity paralleling the kinships of animals may be shown by immune sera. It harmonizes with what we know of the architecture of the native proteins as well as with our whole scheme of natural biological taxonomy in which we find certain fundamental stable features representing a broad series of organisms, and less and less inclusive characteristics which grade down to the minor differences that separate species, varieties and individuals. Nor is it incompatible with what we know of chromosomes and genes. The very fact that heritable grades of a single gene in a given chromosome may occur (*e.g.*, in *Drosophila*) and that one of these variants may in turn be modified gradationally by a series of secondary factors located in other chromosomes suggests the type of organization just discussed.

With the remarkable and abundant evidence of hand-and-glove relationships between unit-characters and chromosomes that has been accumulated in recent years through the painstaking studies of workers on *Drosophila,* not to mention other corroborative work, it seems to me that there is no longer a reasonable doubt that the differentials, whatever they may be, responsible for the distinctiveness of the so-called unit-characters, reside in the chromosomes. And while I have always believed[11] and still believe that for the final outcome the cytoplasm is just as necessary in its way, and must be just as characteristic of the species as the chromosomes are, its distinctiveness must be of a fundamental organismic (probably chemical) type common to the species as a whole,

[11] Bull. No. 2, Univ. of Cincinnati, Vol. III, Ser. II, 1902. *Science,* June 28, 1907. Univ. Cincinnati Studies, Sept.–Oct., 1909. AMER. NAT., XLV, 1911.

since it can be, in fact is, contributed in reproduction almost wholly by one parent, the female. It is apparently a medium which responds specifically to the action of the respective chromosomal incitants, whether these be of maternal or paternal origin. All geneticists agree to-day, I think, that any character of an adult can not be merely the outcome of a unitary germinal antecedent; it is the product of many factors. And ordinarily what we see as a character-difference is probably merely the outcome of a factor-difference in one of the chromosomal cooperants.

In conclusion, let me say first of all that no one more than myself realizes the inadequacy of my present argument as a complete or satisfying theory. The knowledge in the fields on which it is based is as yet far too fragmentary to warrant anything but tentative conclusions. But since various facts seem to me to point toward the view that certain types of immunological reactions, notably the cytolytic, engendered against various somatic constituents may occasionally also affect chemically related substances in the germ, and inasmuch as many other facts lend themselves to such an interpretation without undue violence to scientific credulity, I have felt justified in presenting the whole matter in the form of a working hypothesis.

In the short time remaining I can not enter into the important question of whether or not changes induced in the blood-serum might be instrumental in leading to progressive rather than regressive evolution, and even had I time for such a discussion, there are not sufficient data available to support such a discussion affirmatively. I should like merely to point out in closing that through exercise we can initiate and promote growth in various parts of the soma, we can induce hypertrophy, and in so doing we are in some way leading the protein and other constituents of the cells in question to make more of their own kind of substance, in other words, to reproduce their kind. We do not know what stimulates them to do so, but, in part, it may well be something that is or can be transported in the circulating fluids of the body; and if so, then there exists the possibility that the corresponding germinal representative of such a part, however tenuous the thread of chemical connection, might also be modified in the direction of progressive germinal change.